UNIVERSITY OF GONDAR

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

DEPARTMENT OF CHEMISTRY

Simultaneous determination of paracetamol and *para*-aminophenol using activated glassy carbon electrode

By Takilu Tukuye

Advisor: Dereje Yenealem (M.Sc.)

A Research Thesis Submitted to University of Gondar Department of Chemistry in Partial Fulfillment of the Requirement for the Masters of Science Degree in Chemistry

(Physical Chemistry)

June, 2017

Gondar, Ethiopia

UNIVERSITY OF GONDAR

POST GRADUATE PROGRAM

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

DEPARTMENT OF CHEMISTRY

This is to certify that this thesis prepared by Takilu Tukuye entitled "SIMULTANEOUS DETERMINATION OF PARACETAMOL AND *PARA*-AMINOPHENOL USING ACTIVATED GLASSY CARBON ELECTRODE" has been submitted for examination with my approval as University adviser and submitted and approved for the degree of master of science in chemistry complies with the regulation and meets the accepted standards with respect to originality and quality.

Boards examiners

Approved by

Thesis supervisor/advisor/	signature	Date
Dereje Yenealem (MS.c.)	_____	_____

Examiners

1. Dr. Muluken Aklilu	_____	_____
2. Dr. Molla Tefera	_____	_____

Chair Person

Dr. Madhusudhan	_____	_____

DECLARATION

First I declare that this thesis is my original work and that all sources of material used for this thesis have been dully acknowledged. This thesis has been submitted in partial fulfillment of the requirements for M.Sc. degree at university of Gondar and is deposited at the University library to be made available to borrow under rules of library.

Brief quotation from this thesis is allowed without special permission provided that accurate acknowledgment of source is made. Request for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of Chemistry department when in his, or her judgment the proposed use of the material is in the interest of scholarship. In all other instances, however, permission must be obtained from the author.

Name: Takilu Tukuye signature_____

Place: University of Gondar

Date of submission:_____

ACKNOWLEDGMENTS

First, I would like to extend my sincere thanks to almighty God for helping me on this work and for all his help throughout my life.

Following, I would like to extend my gratitude and heartfelt thanks to my advisor, Dereje Yenealem (M.Sc.), for his guidance, suggestion and correction from the very beginning of the selection of the title of the study to write up the thesis paper.

My appreciable thanks also go to Dr. Meareg Amare, Bahirdar University Lecturer for his incredible support and guidance throughout the laboratory work. I also would like to thanks Dr. Arul Jeevan for providing *para*-aminophenol from India.

I would like to extend my acknowledgment to the Ethiopian ministry of education for sponsoring me to do my masters study and for this work. I would like to extend my gratitude thanks to the Bahirdar University, department of chemistry to give the laboratory facilities to do this work. My grateful acknowledge also go to University of Gondar, department of chemistry for providing some chemicals to accomplish my study.

Finally, I would like to express my sincere thanks to my class met students and my friends who encouraged and helped me during my study. Again I would like to express my appreciation thanks to my family for their prier and moral support up to the end this study.

TABLE OF CONTENTS

Contents **Pages**

LIST OF ABBREVIATIONS AND SYMBOLS

ΔEp	Change in Peak Potential
AGCE	Activated Glassy Carbon Electrode
APAP/NAPQI	Acetyl *Para*-aminophenpl/N-acetyl *para*-quinoneimine
APAP	Acetyl *Para*-aminophenol
CV	Cyclic Voltammetry
DPV	Differential Pulse Voltammetry
Epa	Anodic Peak Potential
Epa	Cathodic Peak Potential
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
LOD	Limit of Detection
LOQ	Limit of Quantification
NPV	Normal Pulse Voltammetry
PAP	*Para*-aminophenol
PBS	Phosphate Buffer Solution
PV	Pulse Voltammetry
SD	Standard Deviation
SWV	Square Wave Voltammetry
TLC	Thin Layer Chromatography
UA	Uric Acid

LIST OF TABLES

Tables **Pages**

LIST OF FIGURES

Figures **Pages**

List of schemes

Schemes **Pages**

ABSTRACT

The study of electrochemical determination of paracetamol and para-aminophenol was investigated by electroanalytical techniques such as CV and DPV on activated glassy carbon electrode. The GCE was activated by applying a potential of 1750 mV for 200 s in time based technique phosphate buffer solution followed by potential cycling 0.0 to 700 mV for six cycles. Activated GCE was chosen for simultaneous determination of paracetamol and para-amino phenol and it showed an excellent electrocatalytic effect and decreases the overpotential than bare GCE. Electrochemical reaction of APAP and PAP at AGCE was found to be reversible involving the transfer of two electron and two proton processes. Phosphate buffer solution pH 6 was used as a solvent and supporting electrolyte for the analysis of standard paracetamol and para-aminophenol throughout the experiment. The peak to peak separation of APAP and PAP is 271.1 mV, which is sufficient for simultaneous determination of the two analytes. The electrochemical reaction of APAP and PAP was adsorption controlled process. The oxidation peak current linearly dependent on the concentration of APAP in the range of 4 μmol L^{-1} to 60 μmol L^{-1} at constant concentration of PAP and concentration of PAP in range of 10 μmol L^{-1} to 240 μmol L^{-1} at constant concentration of APAP. The LOD of APAP and PAP were found to be 1.46 x 10^{-7} mol L^{-1} and 3.24 x 10^{-7}mol L^{-1} respectively and LOQ of APAP and PAP were 4.87 x 10^{-7} mol L^{-1} and 1.08 x10^{-6} mol L^{-1} respectively. Validation of the method was also checked with recovery studies and interferences were also studied.

***Key words**: Paracetamol, Para-aminophenol, Activated glassy carbon electrode, Phosphate buffer solution, Cyclic voltammetry, Differential pulse voltammetry, Limit of detection.*

1. INTRODUCTION

Paracetamol (N-acetyl-4-amino-phenol) is a prominent pain relieving and antipyretic medicine that is promptly absorbed after administration and used for the decrease of fever. It has few side effect and little poisonous quality when used as a part of prescribed measurement. This is because of it can be quickly and totally metabolized by undergoing glucuronidation and sulfation to idle metabolites which are eliminated in urine [1- 2]. It goes about as painkiller by hindering prostaglandin's union in the focal sensory system and relieving fever by steadying the hypothalamic heat regulating center [3]. paracetamol is a compelling and safe pain relieving specialist utilized worldwide for the relief of pain related with arthralgia, neuralgia, cephalagra, headache, backache, and postoperative pain. Recent research proposes that paracetamol may shield from changes prompting to hardening of arteries that cause cardiovascular illness and furthermore now and again used for administration of cancer pain. Paracetamol is a weak anti-inflammatory drug unlike aspirin due to lack of prostaglandin inhibition peripherally in the body. It is synthetic derivative of *para*-aminophenol and is hydrolyzed in an inappropriate condition, such as, high temperatures and acidic or basic media to PAP [4 - 5].

Para-aminophenol (PAP) has been identified in APAP. PAP and its derivatives is the most well-known toxin, highly harmful and mutagenic impact. It also toxic to amphibian life, found in effluent wastes from oil refineries, rubbers, colors, ointments materials and pharmaceuticals generation and processes. PAP has additionally dangerous and allergenic consequences for skin and that it has nephrotoxicity and teratogenic impacts unsafe for people, animals and plants. Its toxicity may induce DNA cleavage in mouse and human lymphoma cells. Subsequently PAP and PAP injection must be constrained. Aminophenols have two oxidizable gatherings OH gathering and NH_2 gathering. These gatherings give more responsive or reactive sites; in this manner aminophenols are fascinating in electrochemical materials. On a basic level, they could demonstrate electrochemical behavior similar to anilines and phenols [6 - 7]. PAP is broadly used as antecedent and intermediate in a variety of chemical synthesis. PAP has been detected in APAP as an impurities or synthetic intermediates and also it is a well known compound that has been used as a redox agent in photography [8].

Several analytical techniques are utilized for the determination of paracetamol and *para*-aminophenol. Large portions of these techniques are boring, tedious, high cost and require pretreatment for the determination. Voltammetric techniques are generally used for the determination of paracetamol and *para*-aminophenol in pharmaceutical arrangements due to their more specific, less expensive and less time-consuming. CV is used to study the electrochemical behavior of APAP and PAP and DPV was used to study the electrochemical determination of APAP and PAP in this study [9].

The fundamental reaction in electrochemical reactions is the transfer of electrons between the electrode surface and molecules in the interfacial region (either in solution or immobilized at the electrode surface). Glassy carbon electrode has become an interesting and widely used electrode material. It exhibits a rather low oxidation rate and a rather high chemical inertness which together with very small pore sizes and a small gas and liquid permeability [10]. In this study activated glassy carbon electrode (AGCE) were used as a working electrode for the simultaneous determination of paracetamol and *para*-aminophenol. Activated glassy carbon electrodes are highly sensitive, fast response and low detection limit. The adsorptive effects result in the improved sensitivity of the voltammetric procedure for a reversible couple formed by a following chemical process after the oxidation paracetamol and *para*-aminophenol [7].

Simultaneous determination of paracetamol and *para*-aminophenol has been studied so far by different researchers using different methods and different electrodes. Typically glassy carbon electrode is not sensitive as activated glassy carbon electrode. The activated Glassy carbon electrodes are used for the reduction in overpotential of the analyte under investigation, increased stability of the electrode response combined with the increment in peak heights, encouraging lower detection limits, expanded specificity, quick reaction, efficient cheap instrument straightforward operation and high affectability. It used to improve the electrochemical response of the biological compounds [8].

A rapid and sensitive voltammetric method for the simultaneous determination of paracetamol and *para*-aminophenol based on activated glassy carbon electrode has been developed. The present study shows that voltammetry with an electrochemically activated glassy carbon electrode is a powerful technique for the simultaneous determination of paracetamol and *para*-

aminophenol in low concentration [10]. Activated GCE surface caused the reversibility of the electrode process by decreasing the overpotential and indicating an improvement in the electrode surface adsorption and highly enhanced peak current. *Para*-aminophenol is the primary hydrolytic degradation product of paracetamol, and has been detected in pharmaceutical preparation of paracetamol as a synthetic intermediate or a degradation product of paracetamol, which can cause teratogenic effect and nephrotoxicity [11]. Therefore, the simultaneous determination of paracetamol and *para*-aminophenol was carried out in this work.

2. LITERATURE REVIEW

2.1 Chemical sensors

A chemical sensor is a device that transforms chemical information, that ranging from the concentration of a specific sample component to total composition analysis, in to an analytically useful signal. The chemical information may originate from chemical reaction of the analyte or from physical property of the system investigated. Advance in the field of sensing has demonstrated that the chemical reaction can be far more selective than the physical interactions [12]. Chemical sensor contains receptor part and transducer part. In receptor part the chemical information is transformed in to a form of energy which may be measured by a transducer. Transducer part is a device capable of transforming the energy carrying the chemical information about the sample in to a useful analytical signal [13].

2.1.1 Activated glassy carbon electrode

A great variety of solid electrodes that can be used as working electrodes have been utilized in various voltammetric techniques throughout the years. Of the wide range of solid electrodes material carbon, platinum, silver and gold are the most well-known solid electrode materials that can be used as working electrode. Carbon based electrodes normally have a more extensive application than the other solid electrodes due to their expansive potential window, low back ground present, rich surface science, minimal effort and appropriateness for different detecting and recognition applications. The surfaces of platinum and gold electrodes are covered with layer of oxides. Owing to this at polarization of the electrodes great residual currents occur also reduction of the oxides and the adsorption and desorption of various compounds is taking place. Chemisorptions and the formation of oxide layer on glassy carbon surface are much lower than on platinum and gold [14]. Glassy carbon electrode is made from a high molecular weight carboneous polymer, often polyacrylonitrile, phenol/formaldehyde resin, etc. Glassy carbon electrode has been exceptionally prominent on account of its excellent electrical and mechanical properties, wide working potential range, extreme chemical substance inertness and moderately reproducible execution [15].

The electrode modification and pretreatments have been broadly used to enhance the electrochemical responses of biological compounds and to build the electrochemical detectors. There are different pretreatment for preparing and activating GCE surface for electrochemical

measurements. Fresh and well-defined electrode surfaces can be prepared by mechanical treatment. Other methods such as laser treatment, irradiation of glassy carbon with ultrasound or activation of glassy carbon using carbon arc have been applied to create a reproducible and active glassy carbon surface. Among these, the electrochemical activation is viewed as an ideal pretreatment technique [16].

Electrochemical pretreatment of glassy carbon electrode is by all accounts a straightforward and less tedious technique in comparison to different systems [17]. Typical carbon electrodes are not sensitive. With a specific end goal to enhance this, surface pretreatment appear to be the most basic matter. The electrode surface alteration with the point of creating electrolytic reaction is offer critical progress [18]. The altered electrodes are used for the reduction in overpotential of the analyte under investigation, increased stability of the electrode response combined with the increment in peak heights, encouraging lower detection limits, expanded specificity, quick reaction, efficient cheap instrument straightforward operation and high affectability [19]. Pretreatment of glassy carbon electrodes with various solvents have critical impact on electrode kinetic, adsorption and capacitance. The moderately substantial surface area of the suspended activated carbon compared with the glassy carbon electrode brings about special adsorption of solvent or electrode impurities on the AGC instead of the bare GC surface [20].

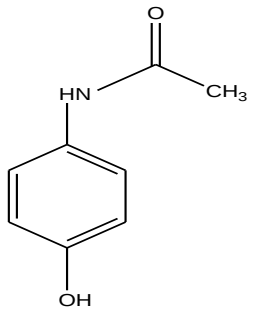
The electrochemical pretreatment simply requires treating the carbon electrode at high potential in a given solvent and electrolyte generates surface functional groups like phenol, carboxyl, carboxylic acid and etc. These functional groups are believed to be the mediators to speed up electron transfer rate so enhance the sensitivity and reproducibility of the electrode [21 - 22].

2.2 Paracetamol and para-amino phenol

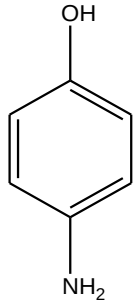
Paracetamol (acetaminophen) is a long established and most generally used antipyretic and pain relieving drug. It is ordinarily used to reducing fever, diminish hack, chilly, and agony including solid throbs, ceaseless torment, headache migraine, spinal pain, toothache and other minor a throbbing painfulness [23]. APAP is the favored other option to headache medicine, especially for patients who can't endure ibuprofen. APAP has little danger at an ordinary measurement be that as it may, overdoses of APAP prompt to hepatic lethality, at times connected with liver and kidney harm and even it can bring about death. Improvement of a technique that could decide

acetaminophen in pharmaceutical and clinical arrangements with no impedance from the other fixing is of a great significance [24].

Several techniques have been accounted for the determination of APAP in organic and pharmaceutical specimens including various high performance liquid chromatography (HPLC) [25], gas chromatography with mass spectrometry (GC–MS) [26], liquid chromatography with mass spectrometry (LC–MS) [27], and spectrophotometry [28]. However, these techniques are costly, tedious and include pre-treatment methodology before identification that makes them inadmissible for a standard examination. Acetaminophen (APAP) is an electroactive compound and voltammetric mechanistic studies for the electrode process of the acetaminophen/N-acetyl-p-quinoneimine (APAP/NAPQI) redox framework are exhibited. Electrochemical technique has attracted in much consideration strategies utilized as detection method for APAP, this is because of quick response, high affectability, and ease examination. Acetaminophen is moderately water and lipid soluble weak organic acid with P^{Ka} of 9.5. Thus it is ionized over the physiological range of pH [23].



APAP

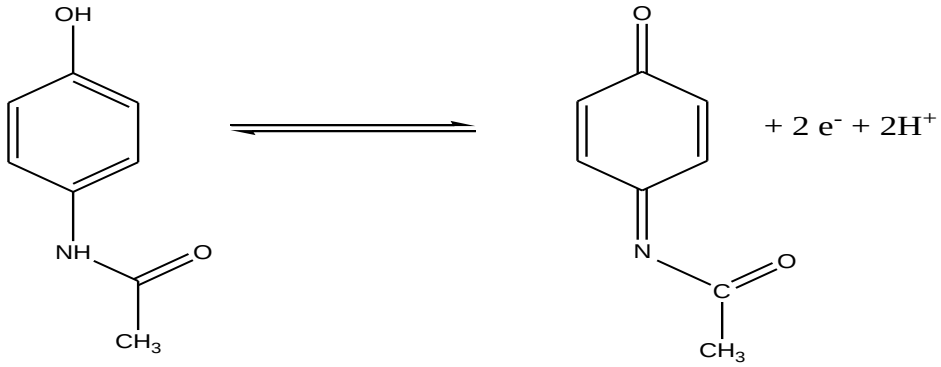


PAP

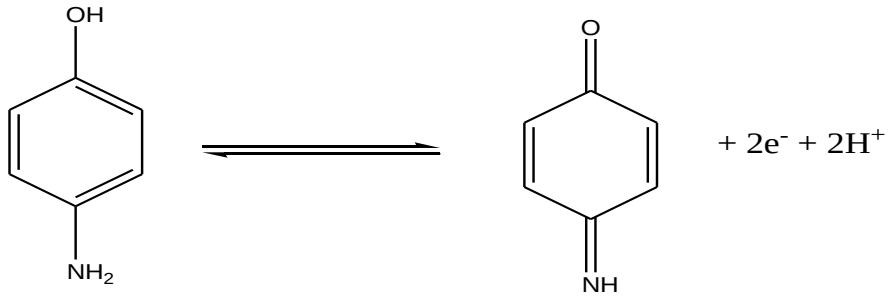
scheme 1: Structure of paracetamol and *para*-aminophenol.

Paracetamol and *para*-aminophenol like many organic substances, undergo an electrochemical mechanism. That is an electron transfer step produces species that under goes a follow-up chemical reaction to produce either electroactive or inactive product. Cyclic voltammetry is an ideal electrochemical method to investigate this mechanism since the fate of the species produced in the forward scan can be ascertained during the reverse scan. The number of electron involved in electrochemical reaction of APAP and PAP can be found from change in potential of pacetamol and *para*-aminophenol on the electrode and the number of proton can be obtained from the plot of formal potential versus pH. The oxidation of both APAP and PAP involves a

two electron and two proton transfer process to form unstable oxidized products as shown on **scheme. 2** [29].



Paracetamol N-acetyl-p-quinoneimine



Para-aminophenol 4-iminocyclohexa-2,5-dienone

scheme 2: Oxidation of paracetamol and *para*-aminophenol.

Para-aminophenol (PAP) is the main impurity which may be present in the preparations containing paracetamol. PAP is a substance of moderately low toxicity, but it is highly toxic and mutagenic effects and induces DNA cleavage in mouse and human lymphoma cells**.** Exposure to PAP and PAP ingestion must be limited, as there are strong evidences that *para*-aminophenol has toxic or allergenic effects on skin and that it has harmful effects for humans, animals, and plants. Therefore very sensitive method of determination is needed [14]. Aminophenols are interesting electrochemical materials. This is because, unlike aniline and other substituted anilines, they have two oxidizable groups (NH_2 and OH) providing more reactive sites. The electrochemical sensing allows fast and selective detection of PAP, avoiding time-consuming procedures and extraction processes, and does not require any complex and expensive apparatus [30].

2.2.1 Electrochemistry of paracetamol and *para*-aminophenol

The simultaneous voltammetric study of paracetamol and *para*-aminophenol was described so far by using glassy carbon electrode coated with gold nano particles and an organophilic layered double hydroxide. The differential pulse voltammetry was investigated in potential range of -200 mV to +800 mV and two well separated oxidation peaks were observed at about 490 mV and 110 mV corresponding to the oxidation of paracetamol and *para*-aminophenol respectively, with limit of detection of paracetamol 1.3 x 10^{-7} mol L^{-1} [11].

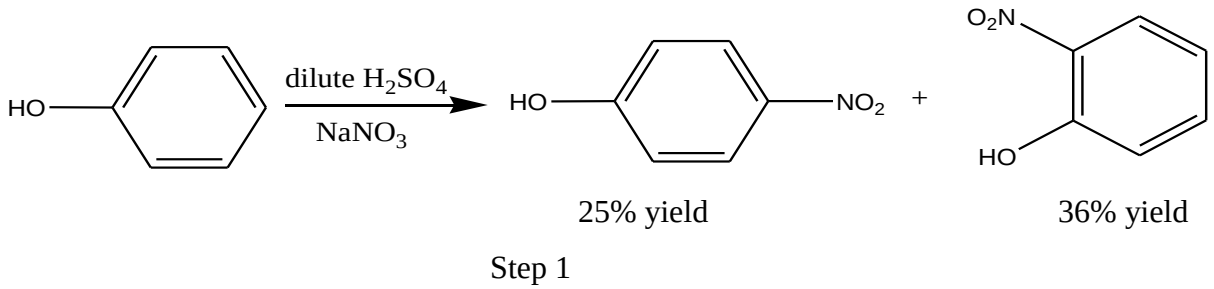
Simultaneous determination of paracetamol and *para*-amonophenol in generic paracetamol tablet using reverse phase high performance liquid chromatography was reported so far [31]. Voltammetric determination was more appreciable by detecting very low concentration, and the effect of interference low in voltemmetic determination compared to chromatography. Simultaneous determination of paracetamol and *para*-aminophenol also studied in the presence of other samples by using HPLC method in paracetamol injection ampoule [24].

By using glassy carbon electrode modified with poly (3, 4-ethylenedioxythiophene) simultaneous determination of paracetamol and *para*-aminophenol was reported. Its differential pulse voltammetric was investigated in potential range of 0.00 mV to 500 mV. The detection limit of 4.048 x 10^{-7} mol L^{-1} and 1.188 x 10^{-6} mol L^{-1} were reported corresponding to paracetamol and *para*-aminophenol respectively [8].

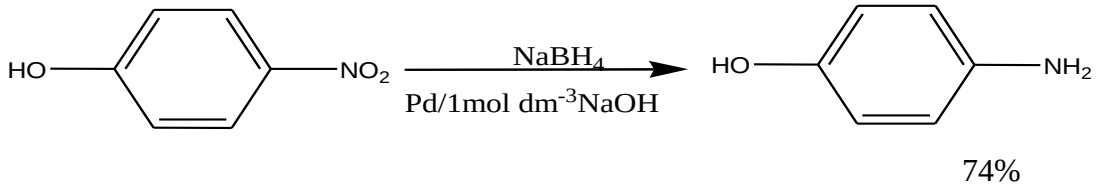
2.2.2. Synthesis of paracetamol and *para*-amino phenol

In the mid of 1980s, Davenport and Hilton reported the pioneering method for the preparation of paracetamol that involve two-step processes. The first step involves reacting of 4-hydroxyacetophenone with hydroxylamine hydrochloride to afford the corresponding ketoxime (4-hydroxyacetophenone oxime) followed by the Beckmann rearrangement in the presence of an acid catalyst, for example fuming sulfuric, hydrochloric, trifluoroacetic, methanesulfonic or *p*-toluenesulfonic acid, amberlyst, nafion, or thionyl chloride in liquid sulfur dioxide. The use of these homogeneous acid catalysts involves boring workup procedures and necessary neutralization of the strong acid media produces undesired wastes. *Para*-aminophenol (PAP) can be produced during the synthesis of paracetamol or may be formed during the storage of preparations [32 - 33].

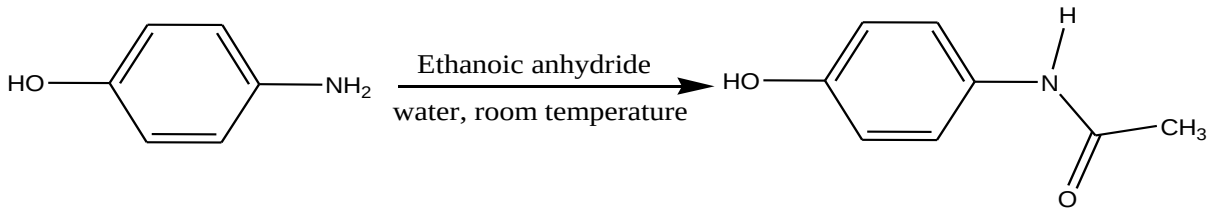
Paracetamol can be formed from phenol in different three steps. By nitrating phenol with sodium nitrate, separating the desired *para*-nitro phenol from the *ortho*- byproduct, and reducing the nitro group with sodium borohydride. The resultant *para*-aminophenol is then acetylated with acetic anhydride. The synthesis of paracetamol and *para*-aminophenol from phenol can be explained in **scheme 3** [34].



Step 1



Step 2



Step 3

scheme 3: Synthesis of paracetamol and *para*-aminophenol.

2.3 Electroanalytical techniques

The development of new strategy equipped for deciding the drug amount in pharmaceutical measurement structures is essential. Electroanalytical methods have been utilized for the determination of an extensive variety of drug compounds with the points of interest that there are in many occasions no requirement for derivatization. These techniques are less sensitive to framework impacts than other analytical techniques. Moreover, application of electrochemistry

incorporates the determination of electrode mechanism. Redox properties of medications can give insights into their metabolic destiny or their pharmacological activity [35].

The term voltammetry refers to a class of electroanalytical techniques, and it is used to designate the current-voltage measurement obtained at a given electrode. The most known characteristics of all voltammetric techniques are that they involve the application of a potential to an electrode and the monitoring of the resulting current flowing through the electrochemical cell. In many cases the applied potential is varied or the current is monitored over a period of time. Voltammetric techniques are considered active techniques due to the applied potential forces a change in the concentration of an electroactive species at the electrode surface by electrochemically reducing or oxidizing it. Voltammetric techniques were useful for simultaneous determination of several analytes [36].

2.3.1 Cyclic voltammetry

Cyclic voltammetry (CV) has turns into a vital and broadly utilized as a part of numerous ranges of electroanalytical chemistry. Cyclic voltammetric techniques, specifically, have regularly been used as a part of fundamental investigations of electrochemical frameworks and in the field of electroanalytical research. It is broadly used for identification of electroactive species, provides electrochemical data on the response rates, the quantity of electroactive species in an answer if each responds at various connected possibilities [37 - 38]. It is done under potentiostatic control. The control parameter is the applied potential; the response signal is the subsequent time course of the current. The fundamental component is the use of cyclic straight voltage slopes with constant raise of changing sign between two voltage limits (inversing potential) [39]. It is rarely used for the quantitative determination however it is generally used for investigation of redox responses and get much information about the chemical reactions occur. The distinct advantage of cyclic voltammetry over the other voltammetric techniques is scanning the potential in both directions; which provides the opportunity to explore the electrochemical behavior of species generated at the electrode. While the applied potential at working electrode in both forward and reverse directions the resulting current is recorded. The output rate in the forward and reverse direction is typically the same. CV can be used as a part of single cycle or multi cycle modes [40 - 41].

The primary instrumental parameter in the cyclic voltammetry is the scan rate ($\nu = dE/t$), since it controls the timescale of the voltammetric experiment. The instrumental yield in cyclic voltammetric methods is a current–potential curve, a cyclic voltammogram. The main features of the cyclic voltammogram are the cathodic and anodic peak potentials, the cathodic and anodic peak current, and the formal (or half-peak) potential. While the half-peak potential (characterized basically as middle between the cathodic and the anodic peak potentials) provides predominantly thermodynamics data, the magnitude of the peak current reveal the kinetics required in the electrochemical response [42].

The shape of the cyclic voltammogram provide information about the sort of the electrode reaction, the number of electrons required in the elementary step of electrochemical change, and in addition about the extra wonders coupled to the electrochemical response of interest, similar to those for coupled chemical reactions or adsorption and crystallization. The electrode reaction is electrochemically reversible when the electron transfer process is much faster than the kinetics of mass transport processes (diffusion). The peak separation can be used to determine the number of electrons transferred and as a criterion for a Nernstian behavior. In this case, the peak separation ΔE_p is defined as follows

$$\mathbf{\Delta E_p = E_{p,c} - E_{p,a} = 2.303 \frac{RT}{nF}} \qquad \mathbf{1}$$

where R is Universal gas constant, T is temperature, n is number of electron transferred and F is Faraday's constant [43].

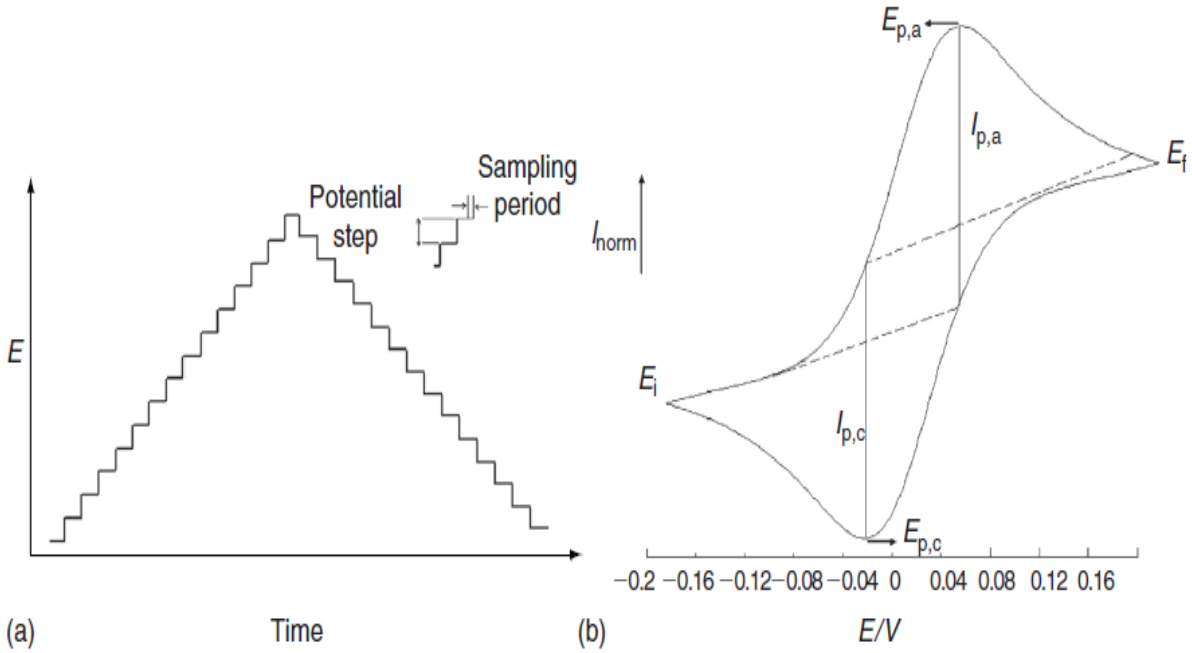


Figure 1: (a) Staircase potential ramp used in cyclic voltammetry, and (b) a cyclic voltammogram simulated for one-electron reversible charge transfer: $E_{p,c}$, cathodic peak potential; $E_{p,a,}$ anodic peak potential; E_i, initial potential; E_f, switching potential; $I_{p,c,}$ cathodic peak current; $I_{p,a}$, anodic peak current.

A cyclic voltammogram shows the presence of all species that undergo oxidation reduction reactions at the working electrode within the limits set by the solvent electrolysis. A redox couple in which both species rapidly exchange electrons with working electrode is termed an electrochemically reversible couple. Such couple can be identified by the formal redox potential by averaging the two peak potentials [36].

$$E^0 = \frac{(E_{pa} + E_{pc})}{2} \qquad 2$$

The peek current for reversible couple (at 25 ^oC) is given by the Randles Sevcik equation

$$I_p = (2.69 \times 10^5)\, n^{3/2} A C D^{1/2} \nu^{1/2} \qquad 3$$

Where n is number of electrons, A is electrode area (in cm^2), C is the concentration (mol cm^{-3}), D is diffusion coefficient ($cm^2\ s^{-1}$) and ν is scan rate (Vs^{-1}) [44].

2.3.2 Pulse voltammetric techniques

The invention of pulse voltammetric techniques was motivated by the fact that by changing the potential and measuring the current in a pulsed manner, a significant discrimination of the charging (non-faradaic) current can be achieved. The basis of all pulse techniques lies in the difference in the rate of the decay of the charging and faradic currents following the potential steps (or pulses). The most important parameters of all pulse voltammetric techniques are pulse amplitude, which is the height of the potential pulse, pulse width, which is the duration of the potential pulse, and sampling period, defined as a time at the end of the potential pulse in which current is measured [45]. There are different pulse techniques, which differ in their potential pulse wave forms, the number of sampling points, and the type of electrode used. Pulse voltammetric technique is appropriate for quantitative analysis due to the discrimination against the charging current that is inherent in these techniques lead to lower detection limit compared to linear sweep technique [46].

2.3.2.1 Normal Pulse Voltammetry (NPV)

This technique uses a series of potential pulses of increasing amplitude. The current measurement is made near the end of each pulse, which permits time for the charging current to decay. It is typically done in an unstirred solution at either DME (called normal pulse polarography) or solid electrodes. The potential is pulsed from an initial potential E_i. The span of the pulse, t, is usually 1 to 100 msec and the interval between pulses regularly 0.1 to 5 sec. The subsequent voltammogram shows the sampled current on the vertical axis and the potential to which the pulse is stepped on the horizontal axis [47].

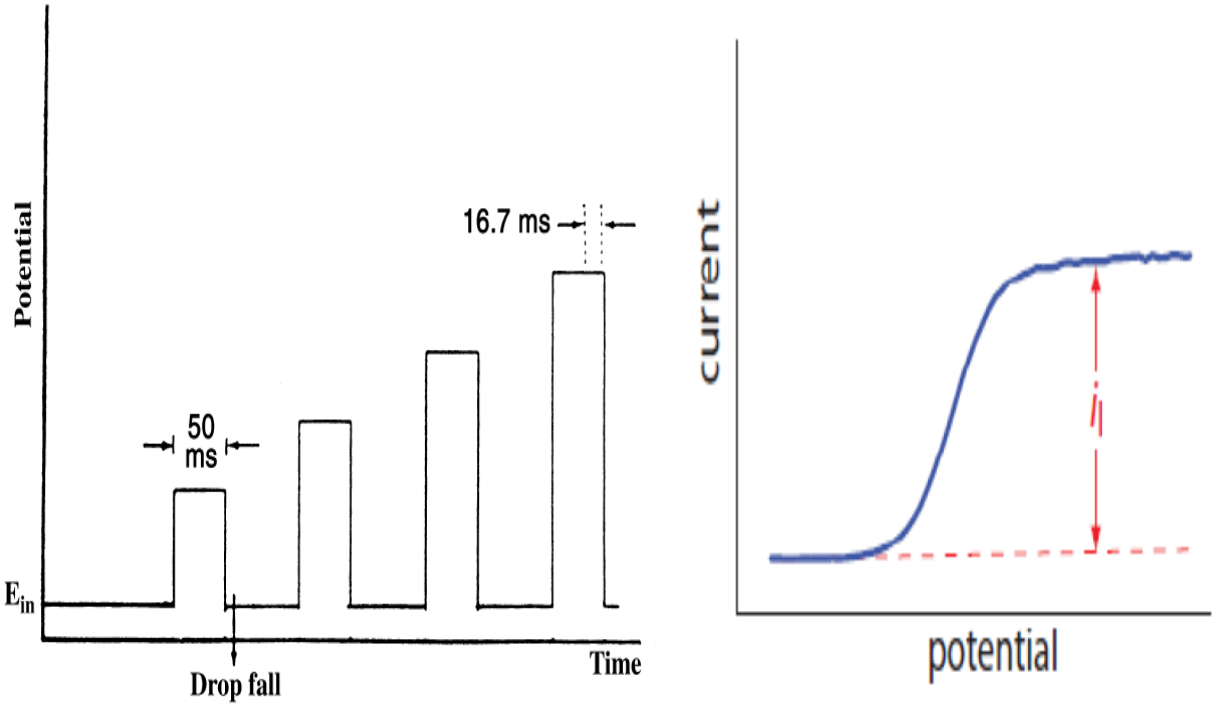


Figure 2: Potential form and resulting simulated voltammogram for normal pulse voltammetry [44].

2.3.3.2 Differential Pulse Voltammetry (DPV)

This technique was proposed by Barker and Gardner. Differential pulse voltammetry is an extremely useful technique for measuring trace levels of organic and inorganic species. DPV can give more noteworthy affectability and more effective resolution and separation of different species. In differential pulse voltammetry fixed magnitude pulses superimposed on a linear potential ramp are applied to the working electrode at a time just before the end of the drop [44]. This technique varies from NPV in light of the fact that every potential pulse is fixed, of small amplitude (10 – 100 mV). Current is sampled twice in each pulse period, once before the utilization of the pulse and at the end of the pulse. These sampling points are selected to allow for the decay of non faradic (charging current). The difference between the current measurements at these points for each pulse [$\Delta i = i(t_2) - i(t_1)$] is determined and plotted against the base potential. At potentials around the redox potential, the difference in current achieves a maximum and decreased to zero as the current becomes diffusion controlled. The current response is in this manner a symmetric peak. The resulting differential pulse voltammogram consists of current peaks, the height of which is directly proportional to the concentration of the

corresponding analytes [48]. Peak shaped response of differential pulse measurements results in improved resolution between two species with similar redox potentials. In various situations peaks separated by 50 mV may be measured. Such quantitation depends on corresponding peak potentials and widths of the peak. The peak shaped response coupled with the flat background current makes the technique particularly useful for analysis of mixtures [44].

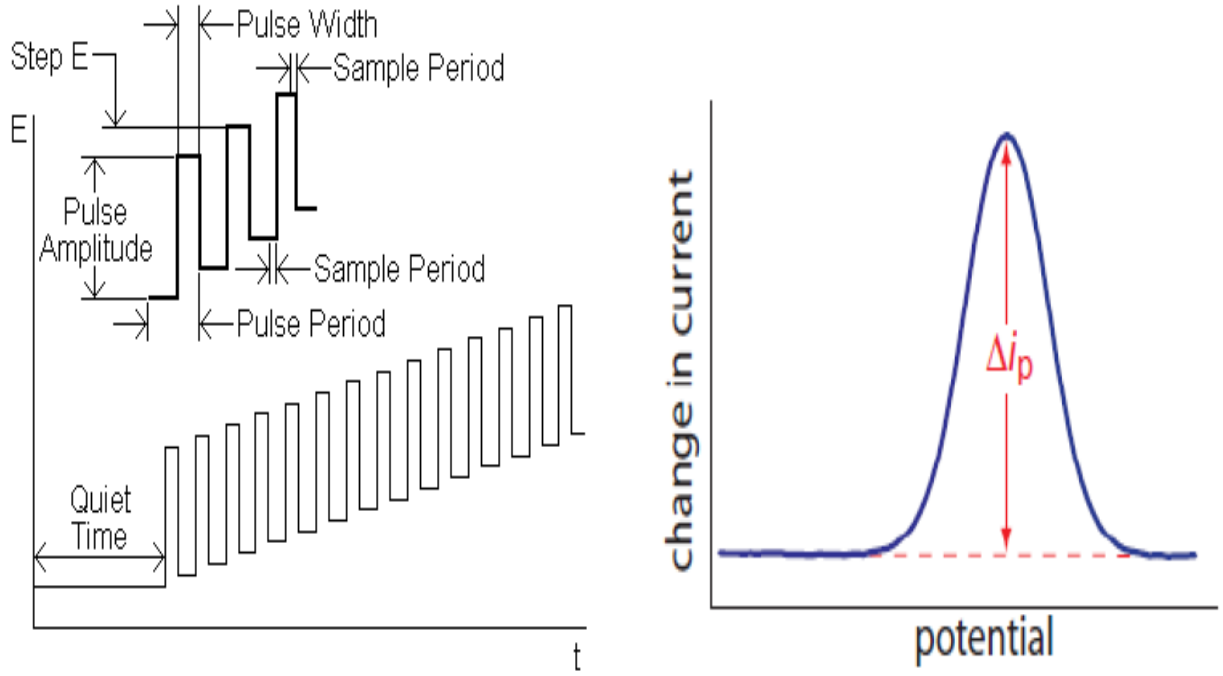


Figure 3: Excitation wave form of differential pulse voltammetry.

The selection of the pulse amplitude and potential scan rate usually requires a sensitivity, resolution and speed. Large pulse amplitudes result in larger and broader peaks. Pulse amplitudes of 25 - 50 mV coupled with a 5 mV/s scan rate are commonly employed. Irreversible redox systems result in lower and broader current peak with inferior sensitivity and resolution compared with reversible systems [48 - 50].

3. OBJECTIVE OF THE STUDY

3.1 General objective

To determine paracemol and *para*-aminophenol simultaneously by CV and DPV using activated glassy carbon electrode.

3.2 Specific objectives

- To investigate the significance of activated glassy carbon electrode for the determination of paracetamol and *para*-aminophenol than bare glassy carbon electrode.
- To study the electrochemical behavior of paracetamol and *para*-aminophenol using CV and DPV at phosphate buffer solution in different pH values and scan rates.
- To identify the type of reaction (adsorption or diffusion) and optimal condition.
- To calculate the LOD and LOQ of paracetamol and *para*-aminophenol using activated glassy carbon electrode.
- To study the effects of interferences on the determination of paracetamol and para-aminophenol.

4. EXPERIMENTAL SECTION

4.1 Instruments and apparatus

The voltammetric experiments were done using CHI 700E, voltammetric analyzer [Bioanalytical Systems (BAS), USA] connected to a personal computer with conventional three-electrode configuration. Electrochemically activated glassy carbon electrode with a diameter of 3.0 mm was used as the working electrode; a platinum electrode served as the counter electrode and silver/silver chloride electrode served as the reference electrode. For preparation of the buffer solutions the pH was measured by a JENWAY model 3510 pH meter. The electronic beam balance (model LA 204) was used to measure the mass of the sample throughout the study.

4.2 Chemicals and reagents

Paracetamol (Addis pharmaceutical factory, Ethiopia), *para*-aminophenol (Godrej industries, India), anhydrous dipotassium hydrogen orthophosphate (BDH, England), potassium dihydrogen phosphate (Sigma-Aldrich, Switzerland), hydrochloric acid (Riedel-deHaen, Germany), and sodium hydroxide (BDH, England) sulfuric acid and panadol (EPHARM) were used as received without any further purification. The stock solution of paracetamol and *para*-aminophenol were prepared by dissolving an accurate mass of the drugs in appropriate volume of the solvent and stored in a refrigerator until used. An aqueous solution was prepared daily of the working days by simple dilution of the stock solution with phosphate buffer. 0.1 mol L^{-1} phosphate buffer solutions (KH_2PO_4 and K_2HPO_4) were prepared by using distilled water. Concentrated hydrochloric acid and sodium hydroxide were used to adjust the pH of the phosphate buffer solution. All chemicals used were of analytical-reagent grade.

4.3 Preparation of the Activated Glassy Carbon Electrode

Glassy Carbon electrode was polished carefully with alumina powder of different particle sizes (1.0, 0.3 and 0.05 μm) and then rinsed with distilled water to remove residual particles. A potential of 1750 mV applied to the GCE for 200 s, sensitivity; 100 μA/V in 0.1 mol L^{-1} KH_2PO_4-K_2HPO_4 buffer solutions. Then the electrode was activated by running cyclic voltammetry from 0.0 to 700 mV for six cycles. The activated electrode was run in cyclic voltammetry and its voltammograms was established [17]. The activated electrode was used as a

working electrode throughout the experiment. The electrolytic effect of AGCE over bare GCE towards paracetamol and *para*-aminophenol was demonstrated by CV between 0.00 mV and 800 mV, and then, the electrochemical properties of APAP and PAP were examined [17, 19, 39].

4.4 Preparation of phosphate buffer and standard solutions of analytes

For analysis of standard paracetamol and *para*-aminophenol 0.1 mol L^{-1} buffer solution of K_2HPO_4 and KH_2PO_4 was prepared and the pH of the buffer was adjusted using concentrated HCl and NaOH. The prepared phosphate buffer solution was used as a solvent and supporting electrolyte throughout the experiment.

4.5 Preparations of calibration curve

The stock solution of paracetamol and *para*-aminophenol was diluted with phosphate buffer solution pH 6 to obtain different APAP and PAP concentrations. Using the optimum conditions described in the experimental section, a linear calibration curve for DPV analysis was constructed in the paracetamol concentration range from $4x10^{-6}$ mol L^{-1} to 6 x 10^{-5} mol L^{-1} at constant PAP concentration ($8x10^{-5}$ mol L^{-1}) and the PAP concentration range from $1x10^{-5}$ mol L^{-1} to 2.4 $x10^{-4}$ mol L^{-1} at constant concentration of paracetamol ($8x10^{-5}$ mol L^{-1}). DPV at pulse amplitude 50 mV, pulse width 50 msec and with potential window of each prepared solution was measured then the calibrations curves were drawn.

4.6 Sample Preparation

Five tablets of the sample were accurately weighed and powdered finely in a mortar. The average weight of each tablet powdered corresponding to the stock solution concentration $1x10^{-3}$ mol L^{-1} was added to phosphate buffer solution 0.1 mol L^{-1} pH 6 in 1000 mL volumetric flask and was shaked till it was dissolved. The solution was centrifuged to ensure complete dissolution and then filtered by Whatman filter paper. The filtrate solution was filled with phosphate buffer solution and further diluted to volume with the same solvent. The diluted solution was contains specified amount of PAP and APAP. From calibration curve concentrations of APAP and PAP were extrapolated and recovery was calculated by dividing the obtained concentration to the spiked [28].

4.7 Electrochemical measurements

Voltammetric determination of APAP and PAP were carried out in a voltammetric cell with 0.1 mol L^{-1} phosphate buffer solution pH 6.0 as supporting electrolyte solution. The electrochemical behavior of APAP and PAP at AGCE was investigated by CV in potential range of 0.00 mV to 800 mV. The electrochemical determination of APAP and PAP was carried out using DPV in potential range of -100 mV to 600 mV. Before each experiment, the AGC was washed with distilled water. Cyclic voltammograms of 0.1 mol L^{-1} PBS was run with a scan rate 100 mV/s until the peak of APAP and PAP disappeared. The APAP and PAP concentrations were obtained by measuring the heights of the oxidative peak currents. The detection limit of APAP and PAP was calculated as three times of the standard deviation of phosphate buffer solution of APAP and PAP divided by the slope of the calibration curve. The limit of quantification of APAP and PAP were calculated as ten times of the standard deviation of phosphate buffer solution of the APAP and PAP divided by the slope of the calibration curve. All electrochemical experiments were carried out at room temperature.

5. RESULTS AND DISCUSSIONS

5.1 Cyclic voltammetric determination of APAP and PAP

The oxidation potential and reduction potential of paracetamol and *para*-aminophenol were studied by using CV on both bare and activated GCE at scan rate of 100 mV/s. Cyclic voltammetric measurements were run from 0.00 mV to 800 mV. APAP was oxidation potential of 502 mV without any reduction peak at bare glassy carbon electrode. **Fig. 4** depicts the cyclic voltammetric response for electrochemical oxidation of 0.1 mmol L^{-1} APAP in phosphate buffer solution on bare and activated glassy carbon electrode. The cyclic voltammograms of APAP shows irreversible electrochemical behavior at bare GCE.

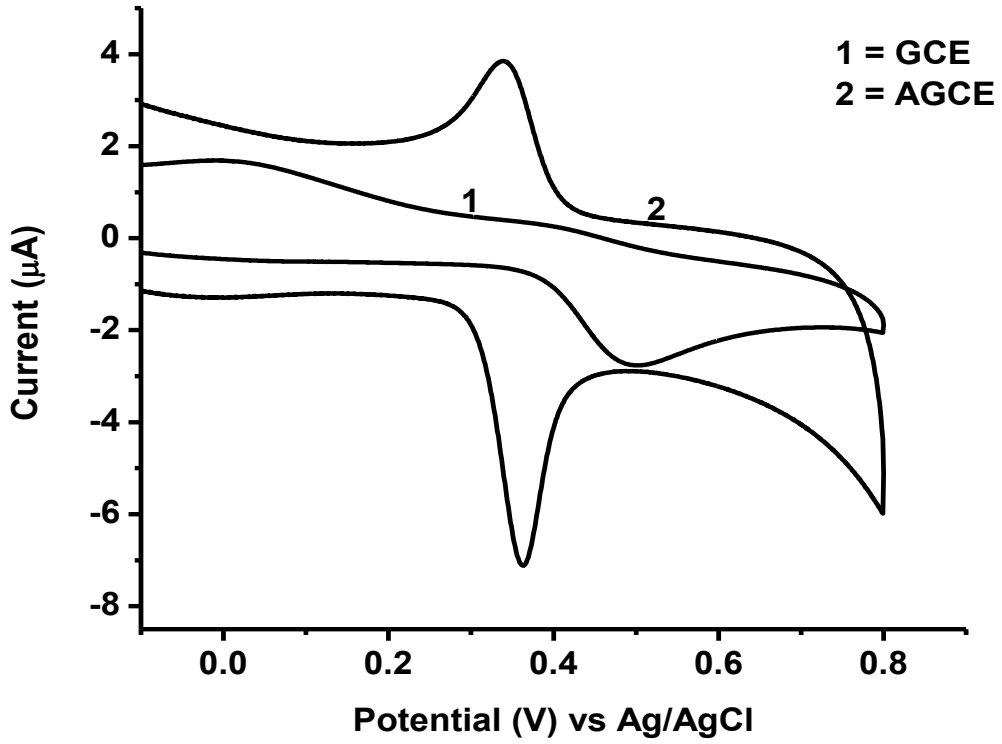


Figure 4: Comparison of cyclic voltammograms of 0.1 mmol L^{-1} of APAP on bare and activated glassy carbon electrode in 0.1 mol L^{-1} PBS.

PAP was oxidized at potential of 155 mV and reduced at potential of was 28 mV at bare glassy carbon electrode. Cyclic voltammograms of PAP shows quasi reversible peak with change in

potential 127 mV on bare GCE in 0.1 mol L^{-1} PBS as it was shown from **Fig. 5**. This shows that slow electron transfer behavior on bare glassy carbon electrode [8].

Para-aminophenol was oxidized at 87.9 mV and reduced at 61.0 mV at AGCE. The oxidation potential shifted to negative from 155 mV to 87.9 mV and reduction potential shifted to more positive from 28 mV to 61 mV. Change in potential (ΔE) decreased to 26.9 mV from 127 mV, i.e. the overpotential decreased with 100.1 mV on activated glassy carbon electrode when compared to bare GCE. Paracetamol was oxidized at 359 mV and reduced at 331 mV with change in potential (ΔE) 28 mV. Therefore according to the reversibility test ΔEp = Eap- Ecp = 59/n mV, both APAP and PAP showed reversible electrochemical behavior at activated glassy carbon electrode. **Fig. 6** shows that the comparison of the cyclic voltammograms of mixture of 0.1 mmol L^{-1} APAP and 0.1 mmol L^{-1} PAP at bare glassy carbon electrode and at activated glassy carbon electrode.

The oxidation potential was shifted to more negative and reduction peak potential shifted to more positive, i.e. peak to peak separation was decreased for both APAP and PAP on activated glassy carbon electrode compared to bare GCE. This shows that there is a reversibility, strong electrocatalytic effect and fast electron transfer rate on activated glassy carbon electrode [8, 17].

Table 1: Summary of peak potentials and peak currents of APAP and PAP obtained from cyclic voltammograms on bare glassy carbon electrode.

Analyte	**Ipa (μA)**	**Ipc (μA)**	**Epa (mV)**	**Epc (mV)**	**ΔE (mV)**
APAP	-2.76	1.7	502	0.93	501.07
PAP	-2.38	2.33	155	28	127

Table 2: Summary of peak potential and peak current of APAP and PAP at activated glassy carbon electrode.

Analytes	Ipa (μA)	Ipc (μA)	Epa (mV)	Epc (mV)	ΔE (mV)
APAP	-7.00	4.00	359	331	28
PAP	-5.08	6.2	87.9	61	26.9

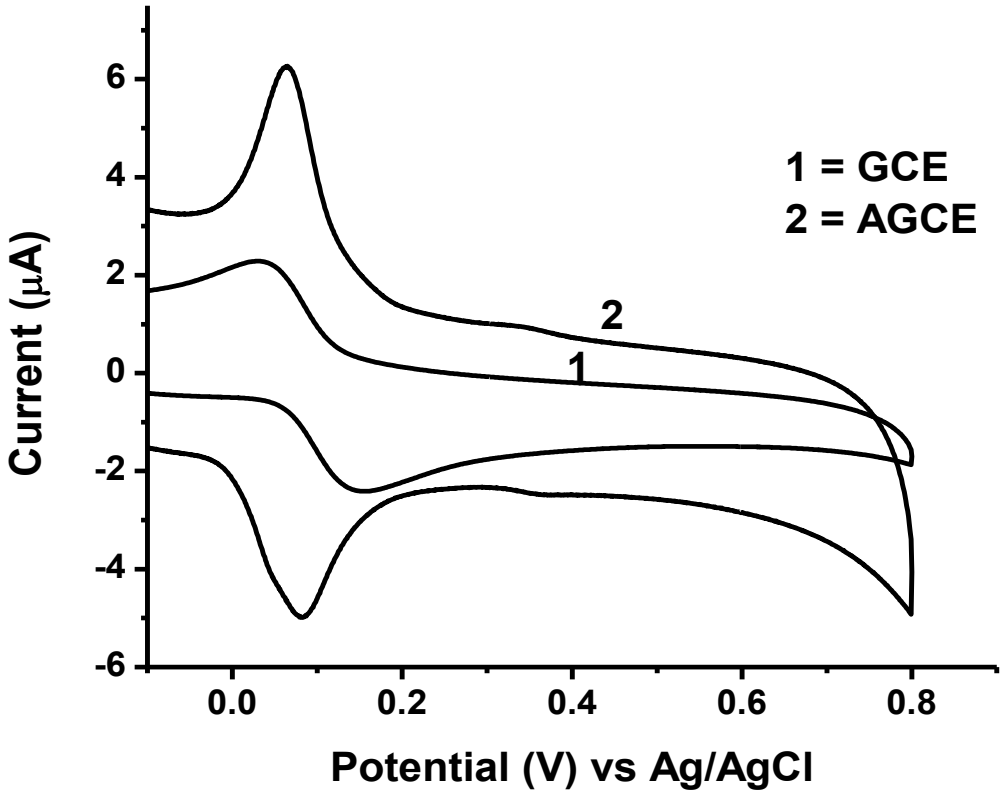


Figure 5: Comparison of Cyclic voltammograms of 0.1 mmol L^{-1} of PAP in 0.1 mol L^{-1} phosphate buffer solution at bare and activated glassy carbon electrode.

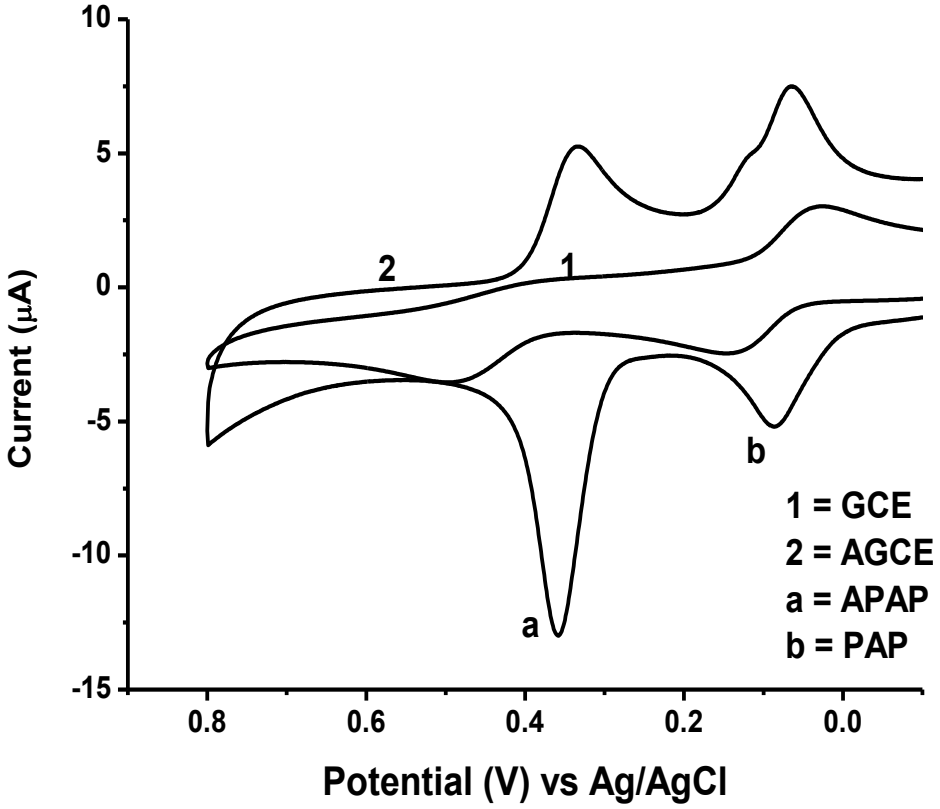


Figure 6: The cyclic voltammograms of 0.1 mmol L^{-1} PAP and APAP at bare (1) and activated glassy carbon electrode (2), oxidation peak of APAP (a) and oxidation peak of PAP (b).

The oxidation peak to peak separation between APAP and PAP was 271.1 mV at activated glassy carbon electrode, this potential gap is sufficient for simultaneous electrochemical determination of APAP and PAP at activated glassy carbon electrode. The peak currents (anodic and cathodic peak currents) are enhanced and the activation over potential considerably decreased on activated GCE than bare GCE.

5.2 Effect of operational parameters

5.2.1 Effect of pH

The effect of solution pH on cyclic voltammetric behavior of APAP and PAP was carefully investigated on activated glassy carbon electrode. The pH value of the solution has a significant influence on the peak current and peak potential of catalytic oxidation and reduction of the APAP and PAP. The studies were carried out in the pH region 2, 4, 6, 7 and 8. The pH increases with potential shifted to more negative. These can also explained by changes in protonation of

the acid-base functions in the APAP and PAP molecules [3]. **Fig. 7** shows the plot of current versus Potential on different pH values and pH increases with anodic current up to pH 6. The high concentration of H^+ hinders the electrochemical oxidation of APAP and PAP due to common ion effect, thus producing a weaker response [8]. Peak current decreased at pH greater than 6 for both APAP and PAP. This is due to the hydroxylation of the mediator at higher pH values. The decrease in the current response is accounted by the electrochemical inactivity of the hydroxylated mediator [51]. Therefore, in order to achieve sensitivity 0.1 mol L^{-1} pH 6 PBS is the most suitable pH for further analytical studies.

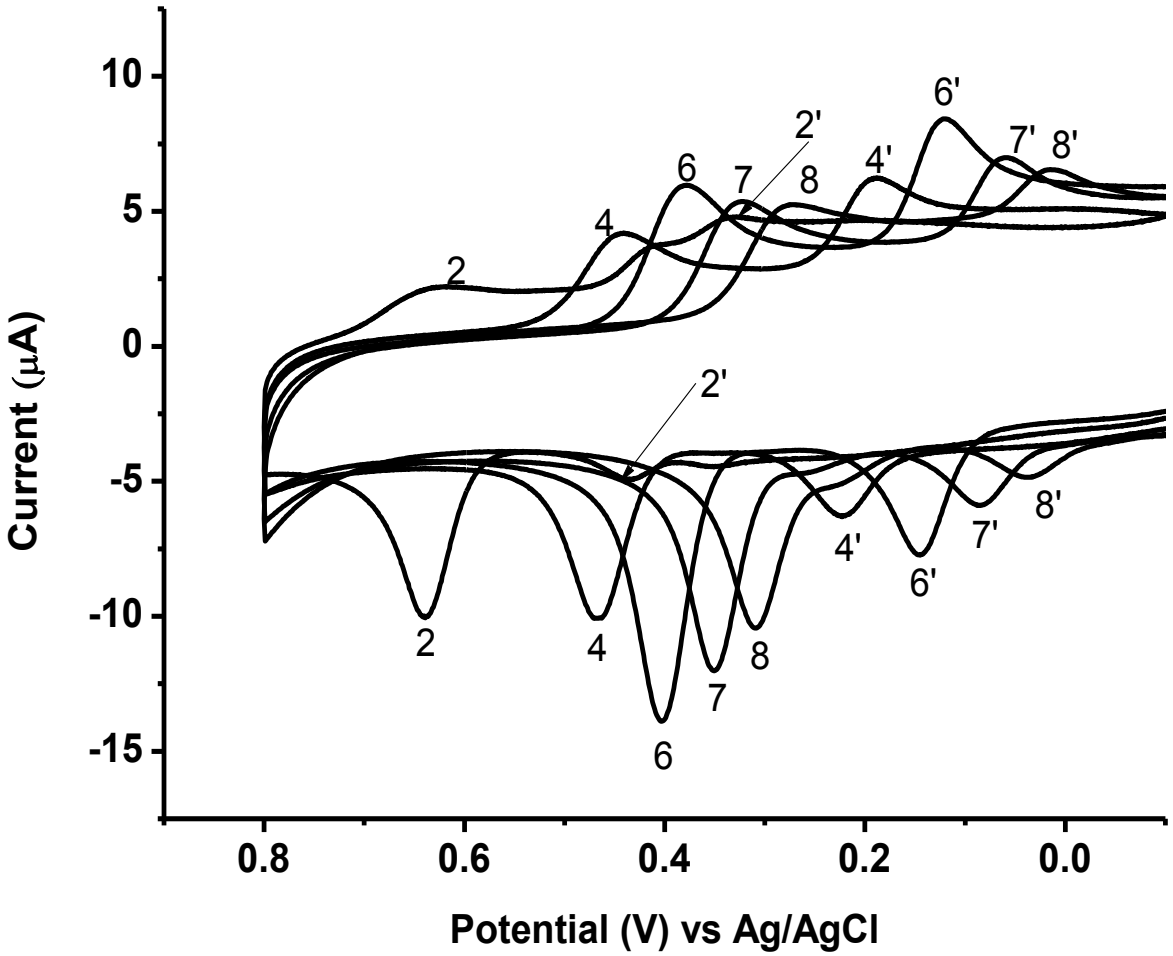


Figure 7: Cyclic voltammogram of different pH values on the peak current and peak potential of 0.1mmol L^{-1} APAP (2, 4, 6, 7 and 8) and 0.1 mmol L^{-1} PAP (2', 4', 6', 7' and 8') in 0.1mol L^{-1} PBS on activated glassy carbon electrode.

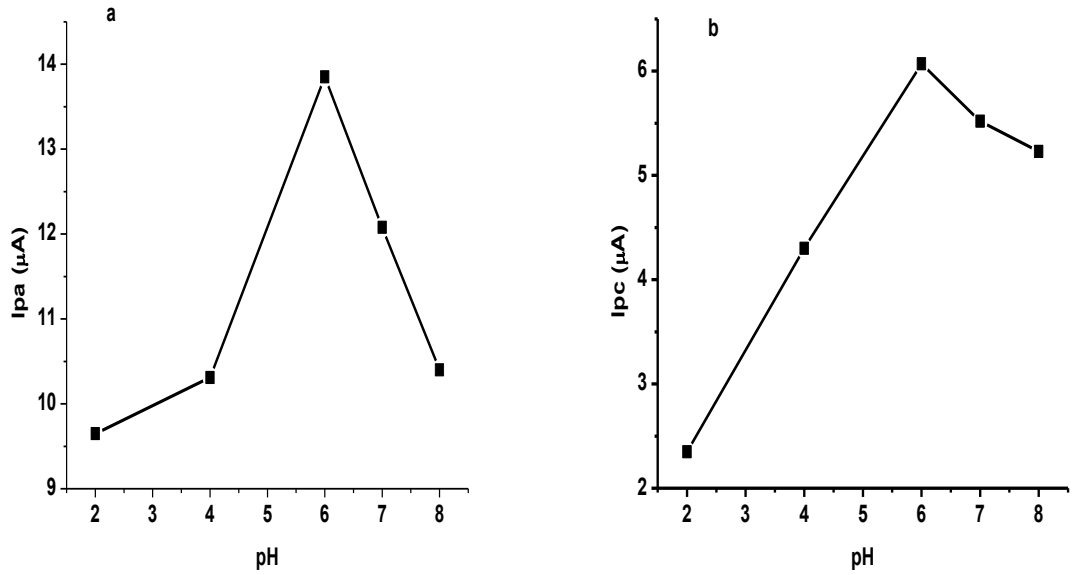


Figure 8: Plot of anodic peak current (a) and cathodic peak current (b) as a function of pH of 0.1 mmol L^{-1} APAP

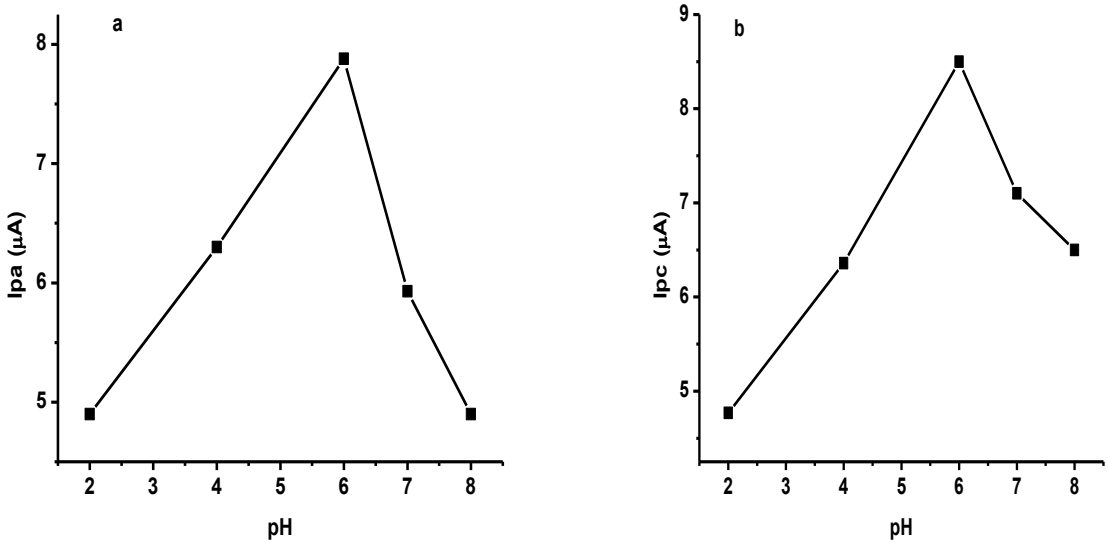


Figure 9: Plot of anodic peak current (a) and cathodic peak current (b) as a function of pH of 0.1mmol L^{-1} PAP.

The number of electron can be calculated by using the equation 1, $\Delta E = 2.303RT/nF$ [8, 52]. In this study change in potential of APAP and PAP were 28 mV and 26.9 mV respectively on activated glassy carbon electrode. The slope value of -53.9 mV pH^{-1} of APAP and -55 mV pH^{-1} of PAP (**Fig. 10** and **Fig. 11**) was close to the theoretical value of -59 mV pH^{-1} according to

Nernst equation, suggesting equal numbers of proton and electron are involved in the redox reaction [2]. Therefore the electrochemical reaction of both APAP and PAP were two electron two proton reversible processes.

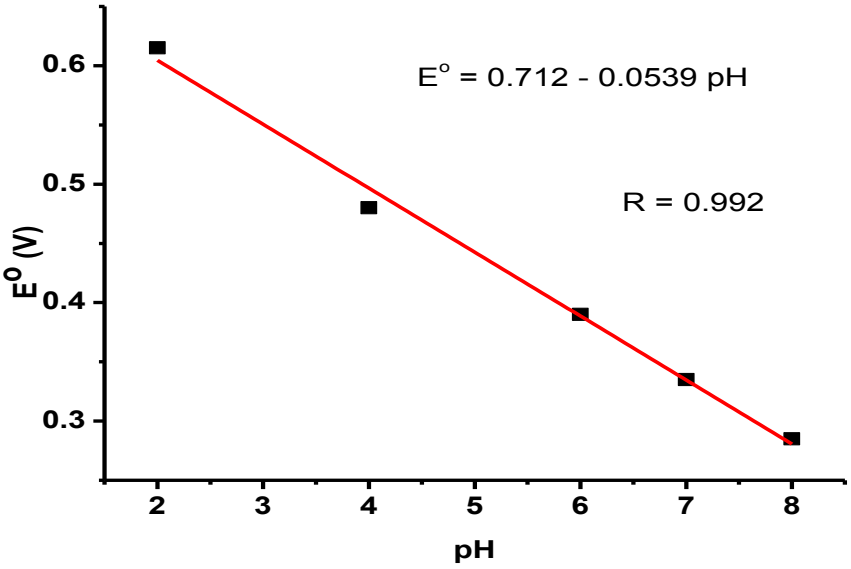


Figure 10: Peak potential versus pH of paracetamol

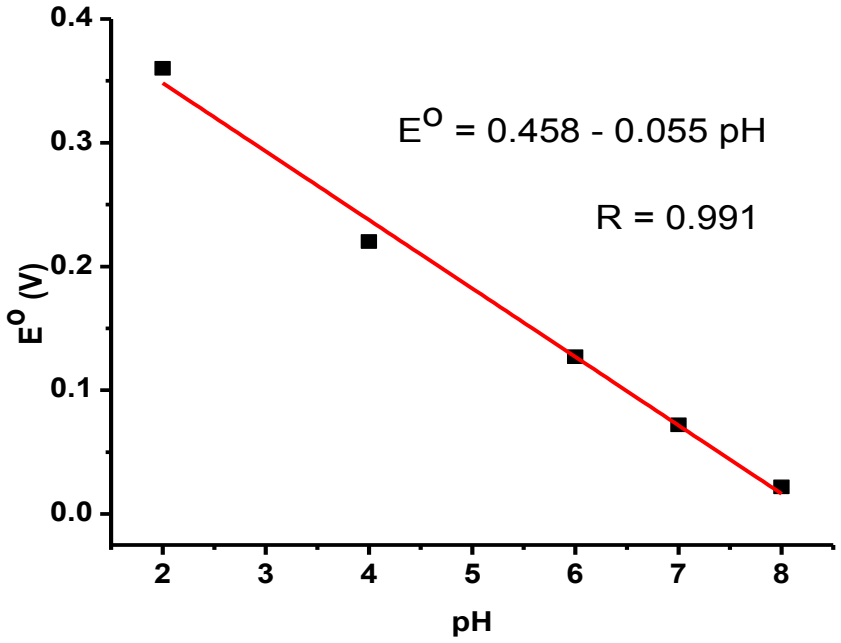


Figure 11: Peak potential versus pH of *para*-aminophenol

5.2.2 Effect of varying scan rate

The effect of varying scan rate (ν) on the electrochemical behavior of 0.1 mmol L^{-1} APAP and PAP at activated GCE as working electrode in 0.1 mol L^{-1} phosphate buffer solution was investigated. The study was carried out in the scan rate range 20 mV s^{-1} - 300 mV s^{-1} over the potential range of 0.00 mV to 800 mV to examine the oxidation and reduction behavior of APAP and PAP. **Fig.12** shows both the oxidative and reductive peak current of APAP and PAP increased linearly with scan rate. **Fig. 12** also shows that influences of scan rate on peak potentials (Epa, Epc). The anodic peak potential (Epa) shifts towards more positive and the cathodic peak potential (Epc) shifts more negative for both APAP and PAP. These indicating that both anodic and cathodic potential are function of scan rate [53].

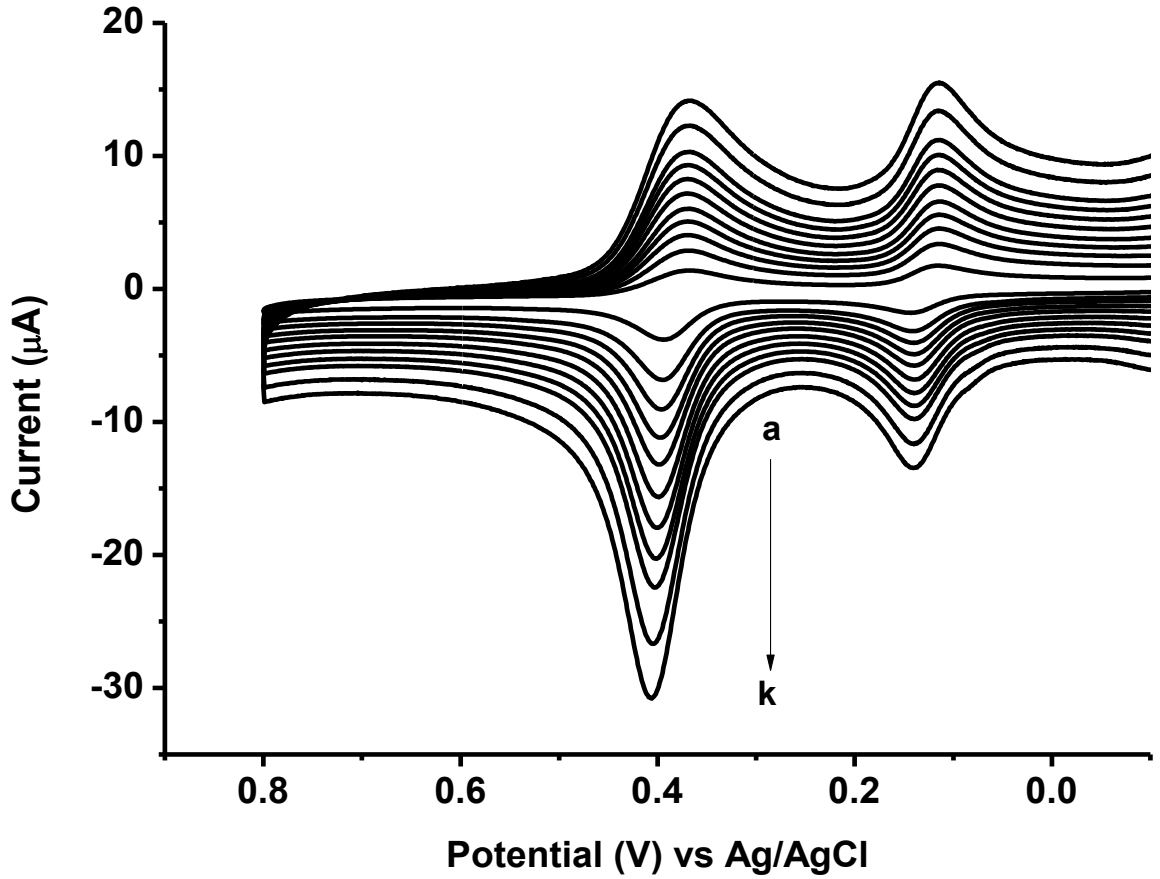


Figure 12: Cyclic voltammograms at the activated glassy carbon electrode in 0.1 mol L^{-1} phosphate buffer solution pH 6.0 at the scan rate of a to k (20, 40, 60, 80, 100, 125, 150, 175, 200, 250 and 300) mV s^{-1} respectively.

In order to investigate whether the oxidation process of APAP and PAP at activated GCE is predominantly adsorption or diffusion controlled process, the correlation coefficients for linear plots of the oxidative peak currents versus the scan rate and oxidative peak current versus the square root of scan rate were compared. The linear relationships between scan rates and peak currents (Ipa and Ipc) were given in **Fig. 13** and **14** with regression equations for APAP Ipc = 1.265 + 0.045*v*, (R = 0.994) and Ipa = -3.33 – 0.094*v*, (R =0.995), for PAP Ipc = 1.62 + 0.048*v*, (R = 0.996) and Ipa = -1.58 – 0.041*v*, (R = 0.998). The linear relationship between square root of scan rates and oxidation peak currents also given for APAP Ipa = -3.57 – 0.99*v*, (R = 0.987) and for PAP Ipa = 3.565 – 1.066*v*, (R = 0.986). The better correlation coefficients for the dependence of oxidative peak currents on the scan rate (R = 0.995) for APAP and (R = 0.996) for PAP than on the square root of scan rate (R = 0.987) for APAP and (R = 0.986) for PAP. This indicated that the oxidations of APAP and PAP at activated glassy carbon electrode were governed by adsorption process. The linear relationship between logarithm of scan rates versus peak currents also calculated with the regression equation for APAP Ipa = -0.855 + 0. 814ln*v*, Ipc = 0.386 - 0.76ln*v* (R = 0.999) and for PAP Ipa = -0.678 + 0.76ln*v*, Ipc = 0.615 – 0.7ln*v* (R = 0.998). The slop of anodic curve for both APAP and PAP was more close to 1. This also indicated that the electrochemical reaction of both paracetamol and *par*-aminophenol at activated GCE were adsorption controlled process [54].

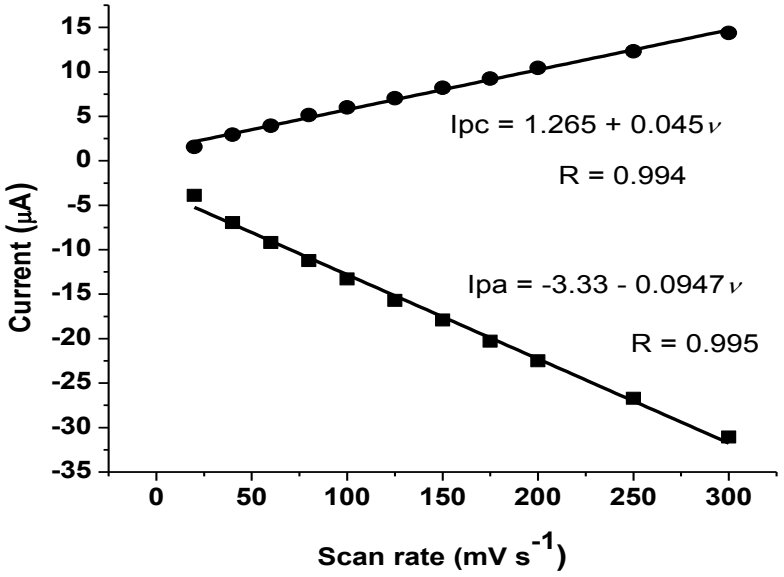


Figure 13: Peak current versus scan rates (20 mV s^{-1} - 300 mV s^{-1}) of 0.1 mmol L^{-1} APAP.

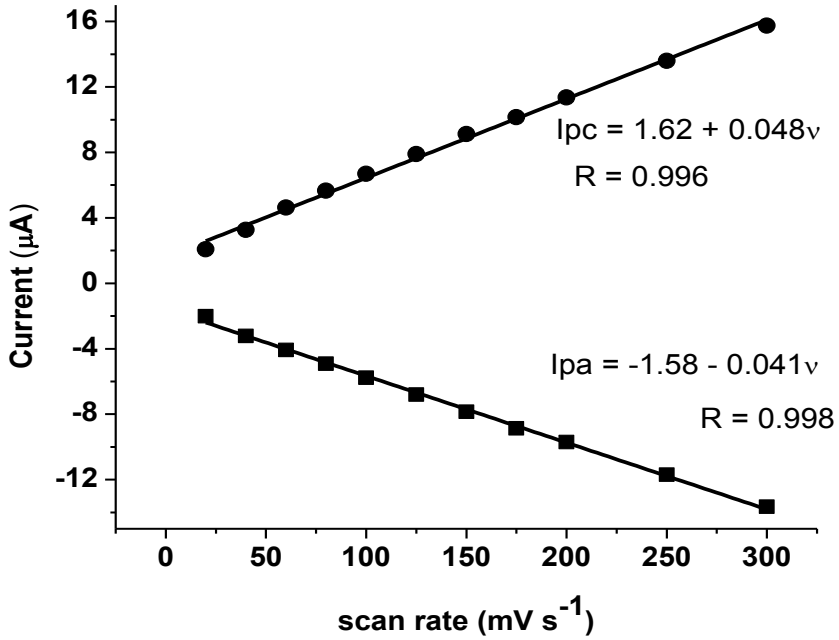


Figure 14: Peak current versus scan rates (20 mV s^{-1} – 300 mV s^{-1}) of 0.1 mmol L^{-1} PAP.

5.3 Differential pulse voltammetric investigation of APAP and PAP

The electrochemical determination of both APAP and PAP were evaluated at activated GCE by using DPV. The DPV was carried out in 0.1 mol L^{-1} phosphate buffer solution pH 6 in the potential range of -100 mV to 600 mV. Two well separated peaks corresponding paracetamol and para-aminophenol were appeared at 378 mV and 116 mV respectively (**Fig. 15**.).

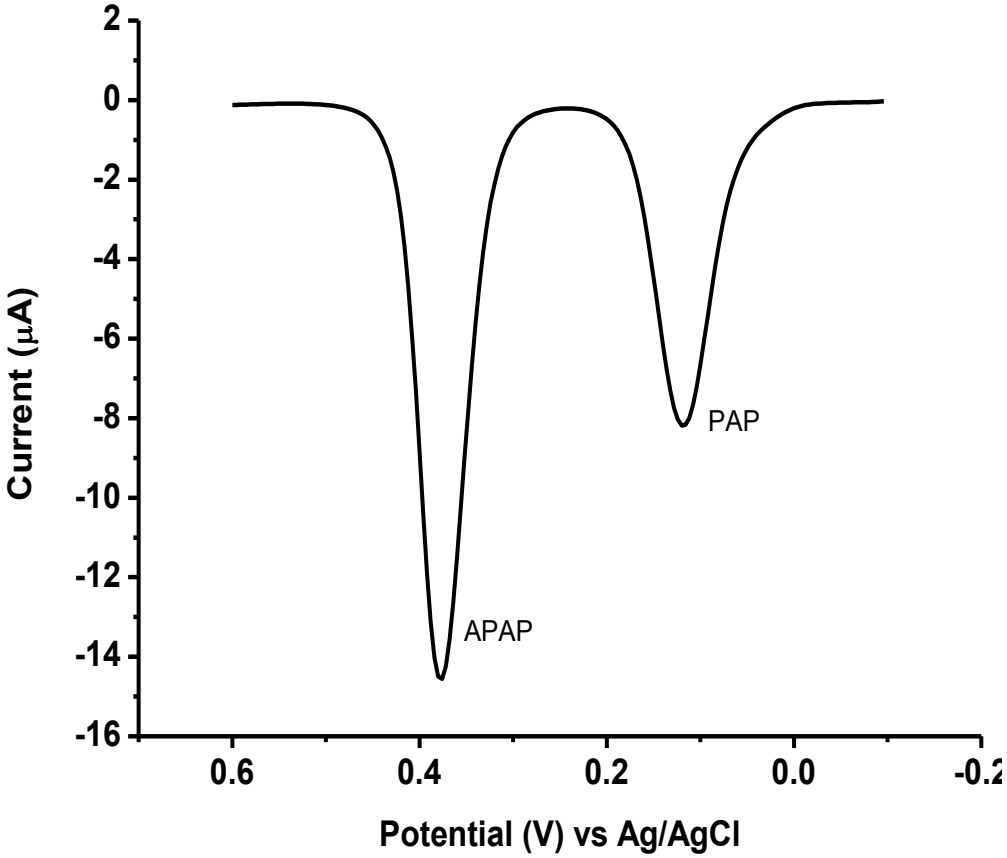


Figure 15: Differential pulse voltammogram of the mixture of 0.1 mmol L^{-1} of APAP and PAP in 0.1 mol L^{-1} phosphate buffer solution on activated glassy carbon electrode.

The stock solution of paracetamol and *para*-aminophenol was diluted with phosphate buffer solution pH 6.0 to obtain different paracetamol and *para*-aminophenol concentration. The DPV technique was selected for simultaneous determinations of APAP and PAP due to its better performance in comparison to the square-wave voltammetry [55]. **Fig.16** depicts the DPV curves of different concentration of APAP ($4x10^{-6}$ mol L^{-1} to $6x10^{-5}$ mol L^{-1}) at fixed concentration of PAP (8×10^{-5} mol L^{-1}) on activated GCE in 0.1 mol L^{-1} of PBS. It was studied with very good linear regression equation of $I_{pa}(\mu A) = -2.3 - 0.487c$ ($R = 0.998$). **Fig. 18**. shows the DPV curves of different concentration of PAP ($10x10^{-6}$ mol L^{-1} to $2.4x10^{-4}$ mol^{-1}) at fixed concentration of APAP (8×10^{-5} mol L^{-1}) on activated glassy carbon electrode. Using the differential pulse voltammetric method, the DPV peak current increased linearly with PAP concentration with good correlation coefficients of $Ipa = -3.49 - 0.27c$ ($R = 0.993$).

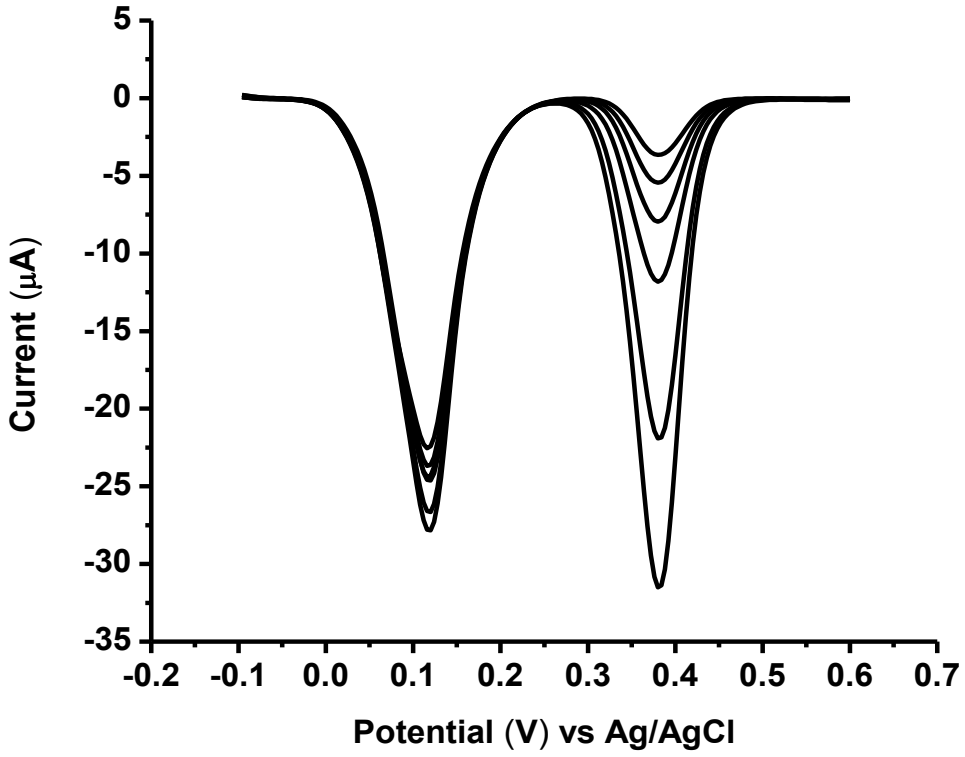


Figure 16: Differential pulse voltammograms of different concentration of APAP (4, 6, 10, 20, 40, 60 μmol L^{-1}) in the presence of 80 μmol L^{-1} of PAP at activated glassy carbon electrode.

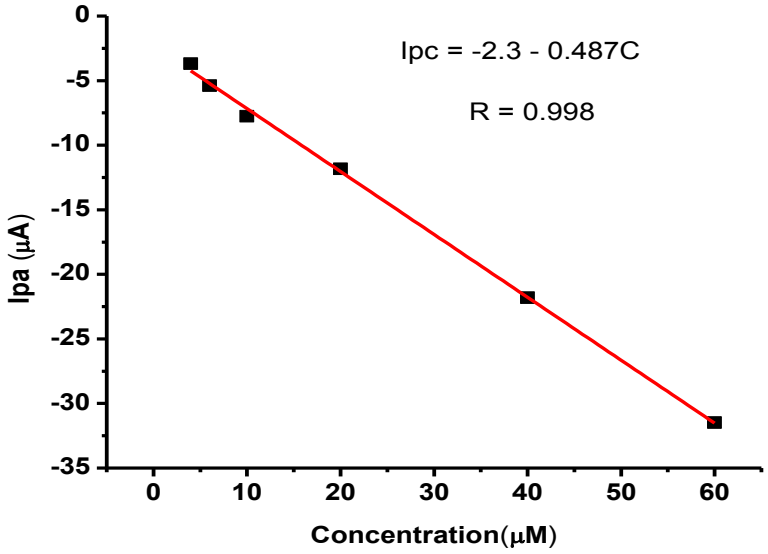


Figure 17: Plot of anodic peak current versus concentration of APAP

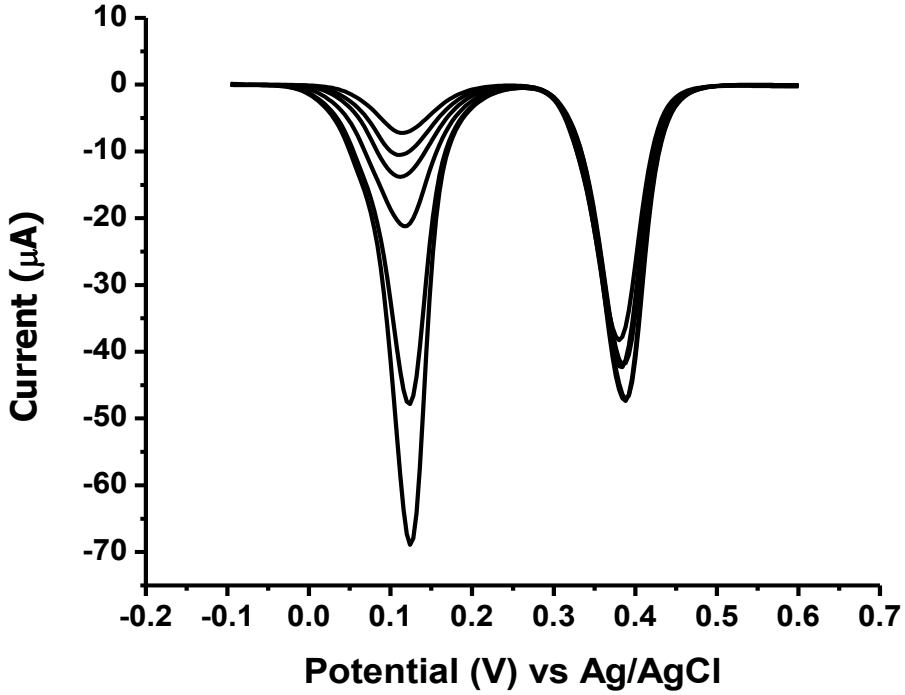


Figure 18: Differential pulse voltammograms of different concentration of PAP (4, 6 10, 20, 40, 80, 160, 240 μmol L^{-1}) in the presence of 80 μmol L^{-1} of APAP at activated glassy carbon electrode.

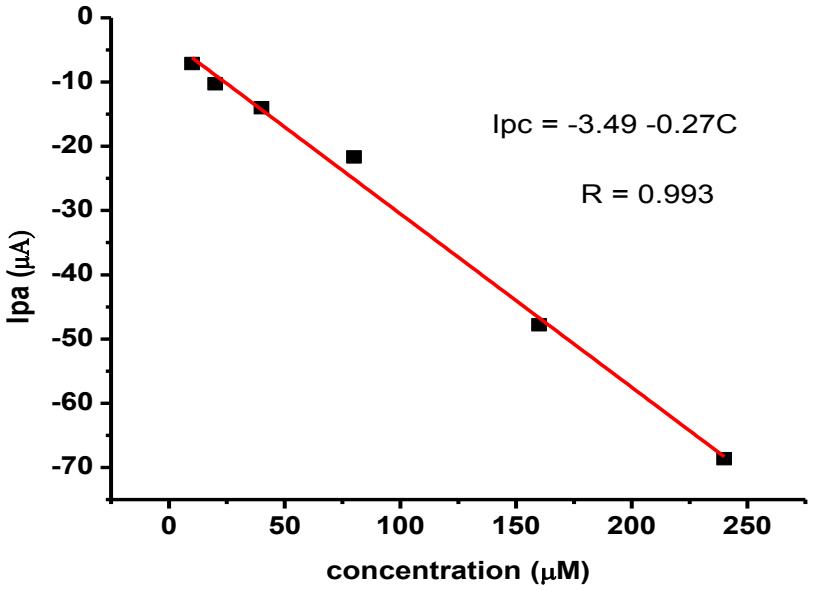


Figure 19: Plot of anodic peak current versus concentration of PAP.

In order to ensure the validation of the procedure limit of detection (LOD) and limit of quantification (LOQ) were studied. The LOD of APAP and PAP were calculated by measuring the differential pulse voltammogram of the phosphate buffer solution eight times (n = 8) without the addition of the analytes. The standard deviation of the eight measurements were 2.34 x 10^{-8} and 2.89 x 10^{-8} for both APAP and PAP respectively. The LOD and LOQ of APAP and PAP were calculated by using equation 4 and 5. The calculated LOD of APAP and PAP were 1.46 x 10^{-7} mol L^{-1} and 3.24 x 10^{-7}mol L^{-1} respectively. Limit of quantification of the APAP and PAP were 4.87 x 10^{-7} mol L^{-1} and 1.08 x10^{-6} mol L^{-1} respectively.

$$\mathbf{LOD = \frac{3SD}{b}} \qquad \mathbf{4}$$

$$\mathbf{LOQ = \frac{10SD}{b}} \qquad \mathbf{5}$$

where SD is the standard deviation of the intercept and b is the slop of the calibration curve.

Table 3: Summary of Analytical parameters of paracetamol and *para*-aminophenol obtained in phosphate buffer solution pH 6 by DPV technique on activated glassy carbon electrode.

Parameters	**Results**	
	APAP	**PAP**
Measured potential (mV)	378	118
Linear concentration range (μmol L^{-1})	4 - 60	10 - 240
LOD (μmol L^{-1})	0.14	0.32
LOQ (μmol L^{-1})	0.48	1.08

5.4 Recovery Test

The percentage recoveries of APAP and PAP in EPHRAM tablet were calculated by using the concentration of APAP and PAP obtained from the signal and the spiked concentration. The results obtained are given in **Table 4**. The percentage recoveries were calculated to be 98.08%

and 107.5% for APAP and PAP respectively. The results showed the validity of the proposed methods for the quantitative determination of *para*-aminophenol and paracetamol in tablet.

Table 4: Determination and recovery of APAP and PAP in commercial tablets (EPHARM) using activated glassy carbon electrode.

Parameters	Obtained results	
	APAP	PAP
Amount unspiked (μmol L^{-1})	40	_
Amount obtained (μmol L^{-1})	38.788	_
Added pure sample (μmol L^{-1})	15	25
Amount spiked (μmol L^{-1})	55	_
Obtained (μmol L^{-1})	53.5	26.88
Recovery (%)	98.08	107.5

5.5 Interference study

To investigate the interference effects, a fixed amount of 1×10^{-4} mol L^{-1} paracetamol and 1×10^{-4} mol L^{-1} *para*-aminophenol was spiked with the same concentration (1×10^{-4} mol L^{-1}) of Uric acid and the DPV was taken in the range of -100 mV to 600 mV. **Fig 20** shows the differential pulse voltammograms of the mixture 0.1mmol L^{-1} of APAP and 0.1mmol L^{-1} PAP and the mixture of the two in the presence of 0.1 mmol L^{-1} of uric acid. The peak to peak separation of paracetamol and uric acid were very small and change in peak current of APAP after addition of uric acid was decreased by 26.44%. Therefore, it is difficult to study the paracetamol in the presence of uric acid.

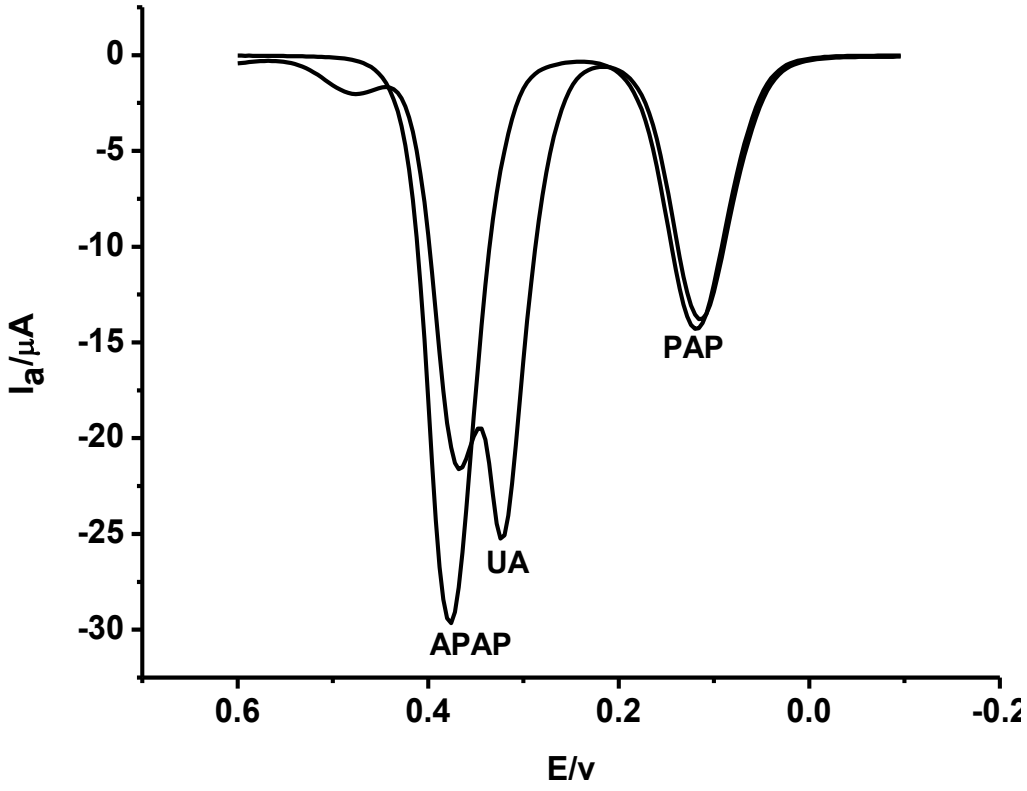


Figure 20: Differential pulse voltammograms of 0.1 mmol L^{-1} APAP, PAP and UA

Table 5: Interference study of APAP and PAP with 0.1 mmol L^{-1} of uric acid

Interferent	Concentration of interferent added to 0.1 mmol L^{-1} APAP and PAP	Recorded signal (Ipa/μA)		Signal change (%)	
		APAP	PAP	APAP	PAP
Uric acid	-	-29.5	-14.33	-	-
	0.1	-21.7	-13.89	26.44	3.07

The presence of 0.1 mmol L^{-1} of uric acid with 0.1 mmol L^{-1} of PAP did not significantly affect the peak current response for the PAP and change in peak current was less than 5% [54].

5.6. Comparision with results of other studies

Table 6: Comparison of simultaneous determination of APAP and PAP with some of the previously reported methods

Method	**Linear range**		**Detection limit**		**Ref.**
	APAP (μmol L^{-1})	PAP (μmol L^{-1})	APAP (μmol L^{-1})	PAP (μmol L^{-1})	
DPV using PEDOT/GC	1 – 100	4 – 320	0.4	1.188	[8]
DPV using GC-coated with gold nano particle	1– 400	0.5 – 200	0.13	_	[11]
HPLC	790 – 1190		1.65	2.29	[24]
RP-HPLC	16 – 130	22.9 – 183	0.39	4.5	[31]
HPLC	530 – 5300	36.6 – 366	_	0.21	[56]
DPV using poly (chromium Schiff base complex) modified electrode at nano-molar levels	0.008 – 0.125	0.008 – 0.133	0.0068	0.0056	[57]
HPLC	1260 - 3010	2.29 – 183	_	0.52	[58]
DPV Using activated GCE	4 – 60	10 – 240	0.14	0.32	This work

6. CONCLUSION

The electrochemical behavior of paracetamol and *para*-aminophenol at activated glassy carbon electrode were studied by using different electroanalytical techniques such as CV and DPV. 0.1 mol L^{-1} Phosphate buffer solution pH 6.0 used as solvent and supporting electrolyte during the experiment. Activated glassy carbon electrode exhibited strong catalytic effect towards electro oxidation of APAP and PAP. The proposed procedure is characterized by many advantages such as short determination time, low cost, wide linear range of paracetamol and *para*-aminophenol concentrations, low detection limits, low quantification limits. The oxidation potential of both APAP and PAP shifted to more negative from 502 mV to 359 mV for APAP and 155 mV to 87.9 mV for PAP and the reduction potential shifted to more positive on activated glassy carbon electrode compared to bare glassy carbon electrode. The catalytic effect was attributed to the appearance of phenolic group and carbonyl group at the electrode surface during the pretreatment step. These groups have catalytic effect on electron transfer mechanisms involving proton transfer. The oxidation peak currents peak potentials of both APAP and PAP was highly dependent on the pH and scan rate. The oxidation of both APAP and PAP involves two protons and two electrons transfer. The linear relationships between scan rates and oxidation peak currents (Ipa) were given with regression equations for APAP (Ipa = -3.33 – 0.094V (R =0.995) and for PAP (Ipa = -1.58 – 0.041V) (R = 0.998). This indicated the adsorption controlled nature of the peak was established. The calibration curve of anodic peak current versus concentration of APAP and PAP showed a linear behavior in a given range 4 μmol L^{-1} to 60 μmol L^{-1} and 10 μmol L^{-1} to 240 μmol L^{-1} with good correlation coefficient of 0.998 and 0.993 respectively. The average recoveries of APAP and PAP in EPHARM paracetamol tablet were 98.08% and 107.5% respectively.

7. REFERENCES

1. Kalaivani, R. and Narayanan, S.S., 2012. Copper hexacyanoferrate-based sensor for anodicdetermination of paracetamol in pure form and in pharmaceutical tablets. *International journal of pharmaceutical and bio sciences*, *3*:128-139.

2. Zidan, M., Tee, T.W., Abdullah, A.H., Zainal, Z. and Kheng, G.J., 2011. Electrochemical Oxidation of Paracetamol Mediated by MgB2 Microparticles Modified Glassy Carbon Electrode. *Journal of Chemistry*, *8*: 553-560.

3. Engin, C., Yilmaz, S., Saglikoglu, G., Yagmur, S. and Sadikoglu, M., 2015. Electroanalytical investigation of paracetamol on glassy carbon electrode by voltammetry. *International Journal of Electrochemical Science, 10*: 1916-1925.

4. Atta, N.F., Galal, A. and Azab, S.M., 2011. Electrochemical determination of paracetamol using gold nanoparticles–application in tablets and human fluids. *International Journal of Electrochemical Science*, *6*: 5082-5096.

5. Filik, H., Avan, A.A., Aydar, S. and Çetintaş, G., 2014. Determination of acetaminophen in the presence of ascorbic acid using a glassy carbon electrode modified with poly (caffeic acid). *International Journal of Electrochemical Science*, *9*: 148-160.

6. Scandurra, G., Antonella, A., Ciofi, C., Saitta, G. and Lanza, M., 2014. Electrochemical detection of p-aminophenol by flexible devices based on multi-wall carbon nanotubes dispersed in electrochemically modified nafion. *Sensors*, *14*: 8926-8939.

7. Suresh, E., Sundaram, K., Kavitha, B., Rayappan, S.M. and Kumar, N.S., 2016. Simultaneous Electrochemical Determination of Paracetamol and Ibuprofen at The Glassy Carbon Electrode. *Journal of Advanced Chemical Sciences*, *2*: 369-372.

8. Tadele, H., 2011. *Simultaneous Determination of paracetamol and para-amino phenol using PEDOT Modified Glassy Carbon Electrode* (Doctoral dissertation, Addis Ababa University).

9. Mbokou, S.F., Pontié, M., Bouchara, J.P., Tchieno, F.M.M., Njanja, E., Mogni, A., Pontalier, P.Y. and Tonle, I.K., 2016. Electroanalytical Performance of a Carbon Paste Electrode Modified by Coffee Husks for the Quantification of Acetaminophen in Quality Control of Commercialized Pharmaceutical Tablets. *International Journal of Electrochemistry, 2016*: 1-10.

10. McCreery, R.L., 2008. Advanced carbon electrode materials for molecular electrochemistry. *Chem. Rev*, *108*: 2646-2687.

11. Yin, H., Shang, K., Meng, X. and Ai, S., 2011. Voltammetric sensing of paracetamol, dopamine and 4-aminophenol at a glassy carbon electrode coated with gold nanoparticles and an organophillic layered double hydroxide. *Microchimica Acta*, *175*: 39.

12. Stetter, J.R., Penrose, W.R. and Yao, S., 2003. Sensors, chemical sensors, electrochemical sensors, and ECS. *Journal of Electrochemical Society*, *150*: 11-16.

13. Hunt, H.K. and Armani, A.M., 2010. Label-free biological and chemical sensors. *Nanoscale*, *2*: 1544-1559.

14. Wyszecka-Kaszuba, E.W.A., 2001. Determination of 4-aminophenol impurities in selected pharmaceutical preparations by HPLC method with amperometric detection. *Acta Poloniac Pharmaceutica-Drug Research, 58*: 325-329.

15. El Ouafy, T., Chtaini, A., Oulfajrite, H. and Najih, R., 2015. Electrochemical studies and cyclic voltammetry of paracetamol at clay modified carbon paste electrode. *Acta Technica Corviniensis-Bulletin of Engineering, 8*: 99-104.

16. Zhao, Q.L., Zhang, Z.L., Bao, L. and Pang, D.W., 2008. Surface structure-related electrochemical behaviors of glassy carbon electrodes. *Electrochemistry Communications, 10*:181-185.

17. Kassa, H., Geto, A. and Admassie, S., 2013. Voltammetric determination of nicotine in cigarette tobacco at electrochemically activated glassy carbon electrode. *Bulletin of the Chemical Society of Ethiopia, 27*: 321-328.

18. Jalali, F. and Hatami, Z., 2016. Fast electrocatalytic determination of methimazole at an activated glassy carbon electrode. *Iranian Journal of Pharmaceutical Research*, *15*: 735-741.

19. Yilmaz, N., Ozkan, S.A., Bengi, U.S.L.U., Senturk, Z. and Biryol, İ., 1998. Voltammetric determination of salbutamol based on electrochemical oxidation at platinum and glassy carbon electrodes. *Turkish Journal of Chemistry*, *22*: 175-182.

20. Zhao, Q.L., Zhang, Z.L., Bao, L. and Pang, D.W., 2008. Surface structure-related electrochemical behaviors of glassy carbon electrodes. *Electrochemistry Communications*, *10*: 181-185.

21. Truc, N.M., Mortensen, J. and Anh, N.B.H., 2008. Electrochemical Preanodization of Glassy Carbon Electrode and Application to Determine Chloramphenicol. *Malaysian Journal of Analytical Sciences*, *12*: 586-592.

22. Li, F., Song, J., Gao, D., Zhang, Q., Han, D. and Niu, L., 2009. Simple and rapid voltammetric determination of morphine at electrochemically pretreated glassy carbon electrodes. *Talanta*, *79*: 845-850.

23. Chitravathi, S. and Munichandraiah, N., 2016. Voltammetric determination of paracetamol, tramadol and caffeine using poly (Nile blue) modified glassy carbon electrode. *Journal of Electroanalytical Chemistry*, *764*: 93-103.

24. Friedman, M., 2014. Chemistry and multibeneficial bioactivities of carvacrol (4-isopropyl-2-methylphenol), a component of essential oils produced by aromatic plants and spices. *Journal of agricultural and food chemistry*, *62*: 7652-7670.

25. Khosroshahi, A.M., Aflaki, F., Saemiyan, N., Abdollahpour, A. and Asgharian, R., 2016. Simultaneous determination of paracetamol, 4-Aminophenol, 4-Chloroacetanilid, Benzyl alcohol, Benzaldehyde and EDTA by HPLC method in paracetamol injection ampoule. *Journal of Pharmaceutical and Health Sciences*, *4*: 61-69.

26. Khorrami, A.R. and Rashidpur, A., 2012. Development of a fiber coating based on molecular sol–gel imprinting technology for selective solid-phase micro extraction of caffeine from human serum and determination by gas chromatography/mass spectrometry. *Analytica chimica acta*, *727*: 20-25.

27. Gopinath, R., Rajan, S., Meyyanathan, S.N., Krishnaveni, N. and Suresh, B., 2007. A RP-HPLC method for simultaneous estimation of paracetamol and aceclofenac in tablets. *Indian journal of pharmaceutical sciences*, *69*: 137-140.

28. Kolivoska, V., Gal, M., Lachmanova, S., Valasek, M., Hromadova, M. and Pospisil, L., 2011. Spectroelectrochemical determination of the electron consumption. *Analytica chimica acta*, *697*: 23-26.

29. Sadok, I. and Tyszczuk-Rotko, K., 2015. New, Simple and Sensitive Voltammetric Procedure for Determination of Paracetamol in Pharmaceutical Formulations. *Insights Anal Electrochem*, *1*: 1-8.

30. El Ouafy, T., Chtaini, A., Oulfajrete, H. and Najih, R., 2014. Electrochemical investigation of 4-aminophenol at natural phosphate modified carbon paste electrode. *Environmental Science: An Indian Journal*, *9*: 352-358.

31. Sornchaithawatwong, C., Vorrarat, S. and Nunthanavanit, P., 2010. Simultaneous determination of paracetamol and its main degradation product in generic paracetamol tablets using reverse-phase HPLC. *J. Health. Res*, *24*: 103-106.

32. Dekamin, M.G. and Nazary, N., 2010. Synthesis of Acetaminophen by Liquid Phase Beckmann Rearrangement of 4-Hydroxyacetophenone Oxime over Nano-Ordered Zn-MCM-41. In *14th Int. Electron. Conf. Synth. Org. Chem*, 14.

33. Jozwiak-Bebenista, M. and Nowak, J.Z., 2013. Paracetamol: mechanism of action, applications and safety concern. *Acta poloniae pharmaceutica*, *71*: 11-23.

34. Ellis, F., 2002. *Paracetamol: a curriculum resource*. Royal Society of Chemistry.1-24

35. Yilmaz, B., Kaban, S., Akcay, B.K. and Ciltas, U., 2015. Differential pulse voltammetric determination of diclofenac in pharmaceutical preparations and human serum. *Brazilian Journal of Pharmaceutical Sciences*, *51*: 285-294.

36. Abrha, B., 2007. *Voltammetric study of paracetamole using glassy carbon electrode.* (Doctoral dissertation, Addis Ababa Univesity).

37. Tefera, M., 2016. *Chemically Modified Electrodes for the Electrochemical Determination of Selected Pesticides and Pharmaceuticals* (Doctoral dissertation, Addis Ababa University).

38. Bernhardt, T.G. and De Boer, P.A., 2003. The Escherichia coli amidase Amic is a periplasmic septal ring component exported via the twin-arginine transport pathway. *Molecular microbiology*, *48*: 1171-1182.

39. Lungu, A., Sandu, I., Boscornea, C., Tomas, S. and Mihailciuc, C., 2010. Electrochemical study of curcumin and bisdemethoxycurcumin on activated glassy carbon electrode. *Revue Roumaine de Chimie*, *55*: 109-115.

40. Farghaly, O.A., Hameed, R.A. and Abu-Nawwas, A.A.H., 2014. Analytical application using modern electrochemical techniques. *International Journal of Electrochemical Science, 9*: 3287-3318

41. Wadhwa, R., Lagenaur, C.F. and Cui, X.T., 2006. Electrochemically controlled release of dexamethasone from conducting polymer polypyrrole coated electrode. *Journal of Controlled Release, 110*: 531-541.

42. Radhia, M.M. and Al-Mullab, E.A., 2016. Voltammetric characterization of grafted polymer electrode self modification with carbon nanotubes (GPESMCNT). *Portugaliae Electrochimica Acta, 34*: 97-103.

43. Kang, X., Wang, J., Wu, H., Liu, J., Aksay, I.A. and Lin, Y., 2010. A graphene-based electrochemical sensor for sensitive detection of paracetamol. *Talanta, 81*: 754-759.

44. Wang, J., 2001. *Analytical electrochemistry.* John wiley and sons.

45. Gulaboski, R. and Carlos, M., 2008. Electrochemical techniques and instrumentation in Food Analysis in Handbook of Food Analysis Instruments.

46. James W. Robinson, Eileen M. Skelly, George M., 2005. Undergraduate instrumentalanalysis, sixth edition, 978-993.

47. Marken, F., Neudeck, A. and Bond, A.M., 2010. Electroanalytical Methods: Guide to Experiments and Applications.

48. Svorc, L., 2013. Determination of caffeine: a comprehensive review on electrochemical methods. *International Journal of Electrochemical Science, 8*: 5755-5773.

49. El Mhammedi, M.A., Achak, M. and Bakasse, M., 2010. Square wave voltammetry for analytical determination of cadmium in natural water using Ca_{10} $(PO_4)_6$ $(OH)_2$-modified platinum electrode. *American Journal of Analytical Chemistry, 1*:150-158.

50. Mirceski, V. and Gulaboski, R., 2014. Recent achievements in square-wave voltammetry. *Macedonian Journal of Chemistry and Chemical Engineering, 33*: 1-12.

51. Filik, H., Avan, A.A., Aydar, S. and Çetintaş, G., 2014. Determination of acetaminophen in the presence of ascorbic acid using a glassy carbon electrode modified with poly (caffeic acid). *Int. J. Electrochem. Sci, 9:*148-160.

52. Shahrokhian. S. and Reyhaneh-Sadat. S., 2011. Voltammetric Determination of Acetaminophen in the Presence of Codeine and Ascorbic Acid at Layer-by-Layer MWCNT/Hydroquinone Sulfonic Acid-Overoxidized Polypyrrole Modified Glassy Carbon Electrode. *International Journal of electrochemistry*,1-10.

53. Wang, J., Shi, Z., Jin, J., Liu, Q. and Zhang, S., 2015. Determination of 4-aminophenol using a glassy carbon electrode modified with a three-dimensionally ordered macroporous film of polycysteine. *Microchimica Acta*, *182*: 823-829.

54. Nikodimos, Y. and Amare, M., 2016. Electrochemical Determination of Metronidazole in Tablet Samples Using Carbon Paste Electrode. *Journal of analytical methods in chemistry*, 2016.

55. Lima, A.B., Torres, L.M., Guimarães, C.F., Verly, R.M., Silva, L.M.D., Carvalho Júnior, Á.D. and dos Santos, W.T., 2014. Simultaneous determination of paracetamol and ibuprofen in pharmaceutical samples by differential pulse voltammetry using a boron-doped diamond electrode. *Journal of the Brazilian Chemical Society*, *25*: 478-483.

56. Crevar, M., Ivkovic, B., Vladimirov, S., Kuntic, V. and Vujic, Z., 2008. Statistical optimization of reverse phase high performance liquid chromatography for the analysis of caffeine paracetamol and its degradation product p-aminophenol. *Acta Chim. Slov*, *55*: 665-670.

57. Kumar, S.P., Giribabu, K., Manigandan, R., Munusamy, S., Muthamizh, S., Padmanaban, A., Dhanasekaran, T., Suresh, R. and Narayanan, V., 2016. Simultaneous determination of paracetamol and 4-aminophenol based on poly (chromium Schiff base complex) modified electrode at nanomolar levels. *Electrochimica Acta*, *194*:116-126.

58. Dung, P.T. and Hai, K.X., 2016. Simultaneous determination of paracetamol, phenylephrine, chlorpheniramine and related compound 4-aminophenol in multi-components pharmaceuticals by high performance liquid chromatography. *Mahidol University Journal of Pharmaceutical Science*, *43*: 37-44.